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THE "VITALITY" OF THE CALCIFIED DENTAL TISSUES

V. PROTECTIVE METAMORPHOSIS OF THE DENTIN[1]

WILLIAM LEFKOWITZ, D.D.S.

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The general manifestations of ageing of tissues are expressed by biochemical and histological modifications. An additional change is the decreased immunity to pathological processes which, paradoxically, does not occur in dentin.

One histological aspect of senescence of dentin may be observed by the examination of ground sections. Recently erupted teeth when observed by transmitted light appear translucent and the dentinal tubules are not clearly discernible (1). *Fig. 1* is a ground section of an upper second molar, post-eruptive age, 10 years. In the main, the dentin both in the crown and root is translucent. In this stage, dentin is highly permeable, the diffusion of dental lymph is maximal and stains and phenol may be introduced into the dentin and transported either to the pulp or periphery (2, 3). Caries is generally most active at this stage, and operative procedures are most painful (4).

In the mature state, the dentin appears opaque when observed by transmitted light. The dentinal tubules appear dark. Beust (5) termed this sclerosis of dentin and observed that it occurred with age. Bodecker (1) noted a similar modification and named the change "Protective Metamorphosis" because it designates a stage of greater resistance to caries, and is allied to the ageing of the teeth. The dentin is also less sensitive to operative procedures. Rushton (6) in examining "dead tracts" of dentin observed that they occur as an age change of the tissue. *Fig. 2* is a photomicrograph of a ground section of a lower second incisor, post-eruptive age, 13 years. The crown dentin appears dark, demonstrating protective metamorphosis. The root dentin is translucent. *Fig. 3* is a photomicrograph of a ground section of an upper second premolar, post-eruptive age, 21 years. There was no peripheral injury to the dentin, the enamel was intact and showed only a slight degree of attrition and the entire body of the dentin appears dark by transmitted light. It may be further observed that the degree of protective metamorphosis is greater in the crown dentin than in the root.

The increased resistance to caries is a matter of concern to the dental profession. Histological examination of metamorphosed dentin has given some indication of the age change that makes it more resistant to caries. There is a

[1] Presented at the 20th annual meeting of the International Association for Dental Research, March 14, 1942, New York, N. Y. (J. D. Res. 21: 302, 1942). Received for publication April 28, 1942.

diminution in or arrest of the diffusion of dental lymph (2). It undergoes degeneration forming a gas. Later, the tubules are occluded by what appears to be calcific plugs. The permeability of metamorphosed dentin is greatly reduced. It has been impossible to introduce stains or phenol into these areas which occur readily in young dentin (2, 3).

Simon and Armstrong (7) who interpreted the appearance of translucent dentin (young dentin) as evidence of catabolism found it significantly lower in organic ash content than in the opaque (metamorphosed) dentin. This is in agreement with unpublished findings of this laboratory that the fluid content of metamorphosed dentin is less than that of young dentin.

The changes of dentin with age can be induced in circumscribed areas by peripheral irritation of the dentin. This may be observed in caries, attrition, abrasion, operative interference or fracture and is confined only to the tract of tubules exposed. The degree of metamorphosis is much more marked in induced metamorphosis but the histological picture appears the same. These tracts which usually overlie secondary dentin are not associated with the ageing process but represent a biologic reaction to irritation (8). *Fig. 4* is a photomicrograph of a ground section of a first incisor at the post-eruptive age of 18. Protective metamorphosis of the crown has begun. At the cervix its development appears retarded. The cervical half of the root is metamorphosed. An area of induced metamorphosis may be observed at I.M. caused by peripheral irritation which also stimulated the formation of secondary dentin, S.D. At P.M. the protective metamorphosis as a result of ageing may be observed. There is no secondary dentin formation. The histological picture of both areas appears the same.

An examination was made of ground sections of 104 teeth of known age. All sections were cut longitudinally as near as possible through the pulp chamber and root canal. Tangential sections which did not include the apical third were rejected. Those cases having coronal caries or fillings sufficiently large to involve most of the coronal dentin also were excluded. They occurred mainly in the younger age groups. Circumscribed areas of opaque dentin induced by caries, attrition and abrasion were disregarded as having any relation to protective metamorphosis as found in older teeth. The degree of metamorphosis was found to increase with age, although no attempt was made to classify it. A specimen showing a slight amount of opacity was considered positive. The specimens were examined by anatomic zones, namely crowns, cervical, middle and apical thirds of the root.

In order to determine the relation between protective metamorphosis and the time factor, the extent of exposure in the mouth was determined for each tooth by using the data of Klein, Palmer, and Kramer (9). They considered a tooth erupted if 1 mm. or more was seen above the gingiva. There being no accurate information on the eruption of third molars, these teeth were excluded from the study. All teeth falling in respective categories of exposure time were grouped together and the percentage of specimens exhibiting metamorphosis in the designated anatomic zones was determined. For purposes of simplicity the average of exposure time for males and females at the age of 15 was used as a standard. Thus it was possible to determine the exposure time of all the teeth.

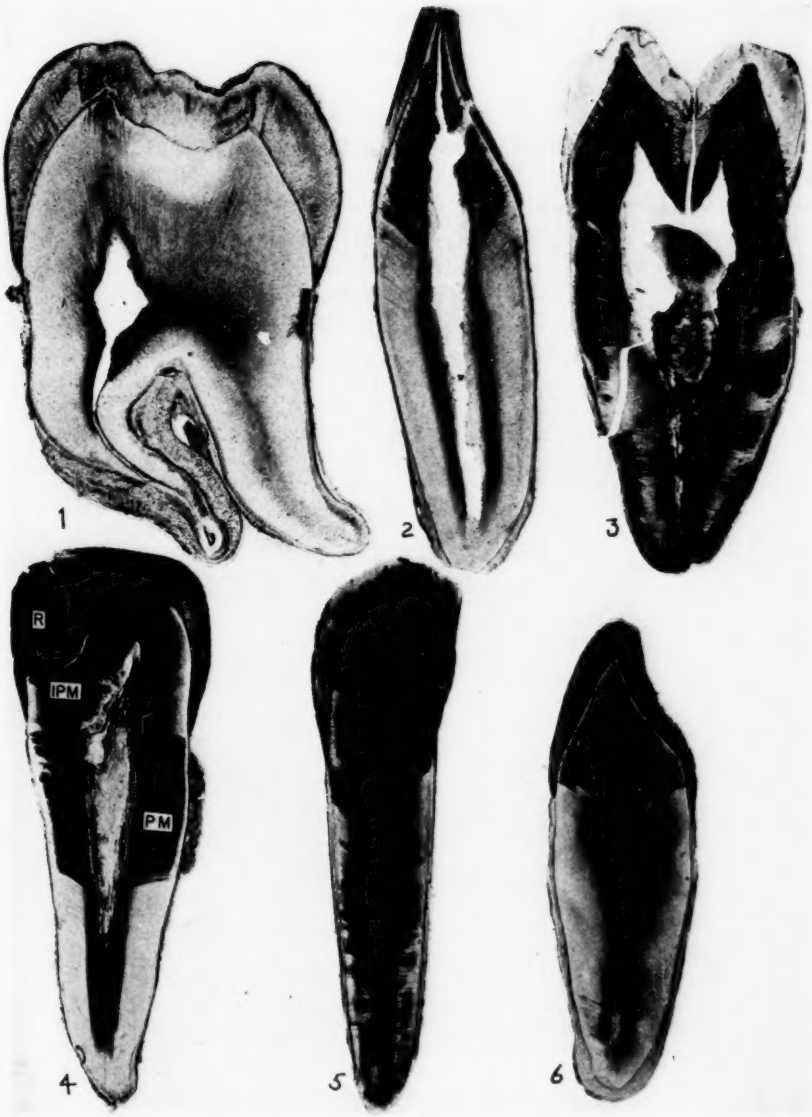


FIG. 1. Ground section, upper second molar—post-eruptive age 10 years. Dentin appears translucent. Slight evidence of beginning metamorphosis. Orig. mag. 9 ×.

FIG. 2. Ground section, lower second incisor—post-eruptive age, 13 years. Protective metamorphosis in crown. Slight change in cervical third of root diminishing toward apex. Orig. mag. 7 ×.

FIG. 3. Ground section, upper second premolar—post-eruptive age, 21 years. Protective metamorphosis throughout body of dentin—more advanced in crown. Orig. mag. 7 ×.

FIG. 4. Ground section, first incisor—post-eruptive age, 18 years. Circumscribed area of induced protective metamorphosis, I.P.M. may be observed under restoration (R) resulting from peripheral, irritation Protective metamorphosis allied to ageing process in crown and cervical half of root. Note similarity of both processes. Orig. mag. 7 ×.

FIG. 5. Ground section, lower second incisor. Intact enamel covered with artificial gold crown. Irregular development of protective metamorphosis in entire body of dentin. Orig. mag. 9 ×.

FIG. 6. Ground section, unerupted canine—age 39. Protective metamorphosis in crown dentin and apical portion of root. Orig. mag. 6 ×.

The distribution of the 104 teeth examined may be observed in Table I.

Table II is a chart showing the number of specimens falling within the levels of exposure time and the number of specimens in these groups exhibiting metamorphosis of the dentin in the respective portions of the tooth.

Table III shows the percentage of teeth with metamorphosis plotted against the exposure time in the mouth, as related to post-eruptive teeth age. The

TABLE I

TOOTH	UPPER JAW	LOWER JAW
	No. of specimens	
First incisor	10	4
Second incisor	2	9
Canine	16	8
First premolar	6	4
Second premolar	9	6
First molar	10	8
Second molar	8	4

TABLE II

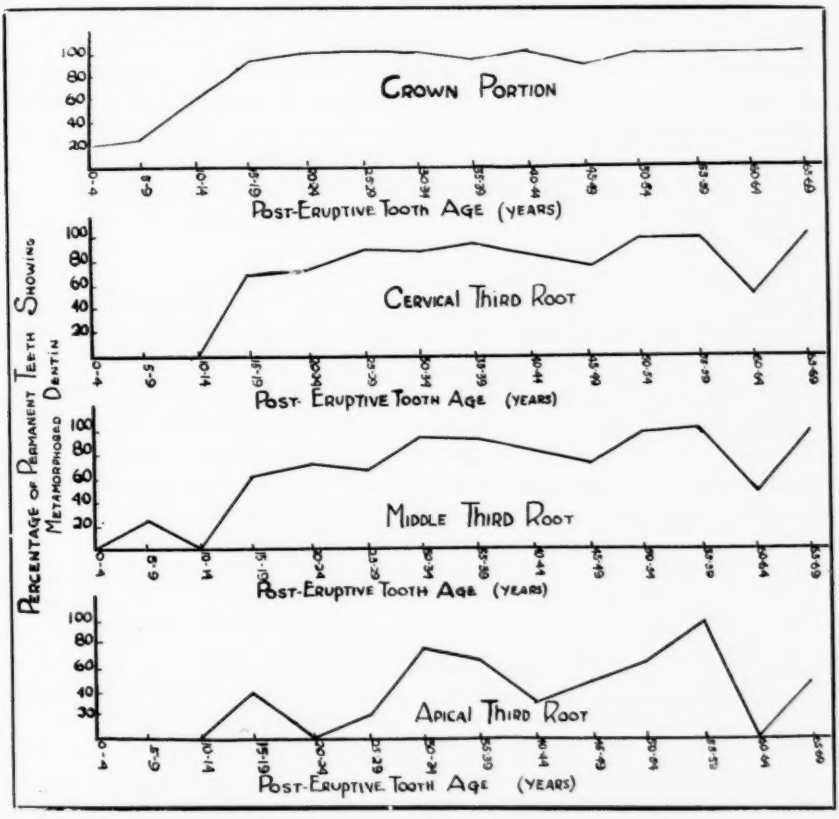
POST-ERUPTIVE TOOTH AGE	NO. OF SPECIMENS	NO. OF SPECIMENS SHOWING PROTECTIVE METAMORPHOSIS IN CROWN	NO. OF SPECIMENS SHOWING PROTECTIVE METAMORPHOSIS IN CERVICAL THIRD OF ROOT	NO. OF SPECIMENS SHOWING PROTECTIVE METAMORPHOSIS IN MIDDLE THIRD OF ROOT	NO. OF SPECIMENS SHOWING PROTECTIVE METAMORPHOSIS IN APICAL THIRD OF ROOT
0– 4	5	1	0	0	0
5– 9	9	2	2	0	0
10–14	5	3	0	0	0
15–19	12	11	8	7	5
20–24	4	4	3	3	0
25–29	10	10	9	7	2
30–34	16	16	14	15	12
35–39	19	18	18	18	13
40–44	7	7	6	6	2
45–49	8	7	6	6	4
50–54	3	3	3	3	2
55–59	2	2	2	2	2
60–64	2	2	1	1	2
65–69	2	2	2	2	0
Total	104				

maximum extent of metamorphosis occurs first in the crowns at about 15 to 19 years after eruption. This level is maintained in the remaining post-eruptive period. In the cervical third, the peak of metamorphosis occurs approximately 10 years later. In the middle third of the root the peak occurs at 30 to 34 years after eruption. The curve for the apical portion differs considerably from that of the crown, cervical and middle thirds of the root. This is undoubtedly due to the irregular development of the metamorphosis at the apical third.

It may be observed that the level of protective metamorphosis in the crown is higher than that of the cervical portion and the level of the cervical higher than that of the middle zone of the root demonstrating that the ageing process proceeds from the crown to the apex. This is not unexpected when one reflects that the formation of the tissue and the progression of protective metamorphosis is harmonious.

The Influence of the Pulp on Protective Metamorphosis: Added to this report are observations on 3 unerupted teeth. There can be no doubt that unerupted

TABLE III



teeth are excluded from the influences to which functioning teeth are subjected. Nevertheless, the ageing process, as manifested by protective metamorphosis, progressed normally. *Fig. 6* is a photomicrograph of an unerupted canine removed from a patient 41 years of age. The dentin exhibits a marked senescent change in the crown. Metamorphosis of the root is much milder.

Another group of teeth included in this study were 9 teeth with artificial crowns. *Fig. 5* is a section of a lower second incisor, post-eruptive age, 31 years. The enamel was intact over the entire crown. The dentin exhibits the normal development of protective metamorphosis being most advanced in the crown and extending throughout the root.

In order to study the direct influence of the pulp on the ageing process of dentin, an examination is now under progress for pulpless teeth. A preliminary report indicates the arrest of protective metamorphosis after pulp removal and that with continued retention a pulpless tooth no longer undergoes an ageing process. The data will be published when sufficient cases are secured.

This finding should not be confused with a previous report (10) on the permeability of pulpless teeth which is greatly increased immediately after pulp removal. Later the permeability is greatly reduced.

Another important observation made in the examination of ground sections confirms the statement of Bodecker (4) that immature or young dentin is found in teeth most susceptible to caries. It was observed, that while protective metamorphosis has a fairly definite age relation and progressive character, there arose, in this examination, an occasional specimen in which this physiological change did not conform to the general age pattern. Those teeth which showed a lack of metamorphosis for their post-eruptive age were highly carious. These cases were responsible for the drop in the graphs at ages 45 to 49 and also in the root dentin at 60 to 64 years.

SUMMARY AND CONCLUSIONS

Biochemical and histological changes indicate that protective metamorphosis of dentin is a manifestation of the ageing process of the tissue. Its occurrence follows a general pattern of proceeding from the crown to the apex. The coronal dentin changes at 15 to 19 years after eruption, cervical dentin 25 to 29 years after eruption and the middle third at 30 to 34 years after eruption. The occurrence in the apical third is irregular. The process was observed in erupted, unerupted and crowned teeth. Preliminary examination suggests that the process of ageing of dentin is arrested upon pulp removal.

Protective metamorphosis may be induced by peripheral irritation (caries, attrition, abrasion) but the primary cause seems to be related to pulpal changes.

Grateful acknowledgment is made to Dr. B. L. Feldman for the statistical report.

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CHANGES IN THE ASH, WATER, AND ORGANIC CONTENT OF PIG ENAMEL DURING CALCIFICATION[1]

MARTIN DEAKINS

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Dental enamel increases markedly in hardness between the time of its deposition and its eruption. Apparently, the hardening is caused by a gradual infiltration of the enamel matrix with inorganic salts in the form of apatite. Whether the hardening is due to the *amount* of inorganic material, to an orientation of the apatite crystals, or to both is not clearly understood. A study of the quantitative changes in the mineral content during development might, therefore, help to resolve these factors which possibly play roles in the hardening process.

Since enamel contains water and organic matter in addition to inorganic salts, it is further possible that changes in concentration of all 3 constituents are associated with the increased hardness. In other words, as enamel becomes hard the amount of inorganic salts may increase while the organic content may or may not change; the influx of inorganic material might be compensated for by a concomitant loss of water. It seems advisable to investigate these possibilities by a method designed (3) to show variations in each constituent independently of the others.

METHOD

The choice of a method for measuring changes in water, inorganic, and organic matter of developing enamel is determined by 3 factors, 1, the density of enamel changes during calcification, 2, the densities of the 3 constituents are all different, and 3, all the constituents may vary in amount simultaneously. Where these conditions obtain, the usual percentage-of-weight analyses are inadequate and misleading. For example, on a percentage basis, an increase of inorganic material having a high density automatically results in an apparent decrease of organic matter, whether the specific amount (mg./cu.mm.) changes or not (1, 2, 3, 4).

This difficulty can be avoided by referring all determinations to volume instead of dry weight. A unit of volume is constant and independent of densities of either the constituents or the sample as a whole. If the samples of enamel and their constituents are reduced in this manner to the common factors, mg. per

[1] Presented at the 20th General Meeting of the International Association for Dental Research, New York, March 14–15, 1942. (*J. D. Res.* **21**: 321, 1942.) Received for publication June 11, 1942.

mm.^{3}, then one may plot the specific weight of any constituent against that of the whole and thus follow the course of each.

Gross examination of the teeth in this study revealed that the degree of hardness varied in different areas of the enamel, increasing generally toward the mesial cusp. Enamel from gingival areas was soft enough to be cut easily with a scalpel while that of the cusps approached the final hardness, the mesial cusp being hardest of all. The subsequent analyses showed increases in specific gravity and inorganic content in the same direction. Therefore, in this investigation, we have employed the density (specific gravity) of enamel as a measure of calcification and the chemical analyses are all referred to it.

EXPERIMENTAL

Three unerupted second molars, at about the same stage of development (*fig. 1*), were extracted from the heads of young pigs while still fresh. From

FIG. 1. Radiograph of pig's mandible showing unerupted molar (x)

various surfaces and cusps of these teeth 45 samples of enamel were removed with a scalpel and the hardness roughly determined with a probe. Each sample (2–10 mg.) was blotted with a moist chamois to remove visible water from the surface and then weighed on the micro-balance. The density of the sample was determined, while still wet, by flotation in a mixture of bromoform and petroleum ether. Next, it was washed with acetone, dried 3 hours or more at 110°C., reweighed, ignited for 10 minutes at about 700°C., cooled, treated with an excess (1 drop) of concentrated HNO_3, and dried in the oven at 110°C.[2] When dry, the ash was again ignited at 700°C. for 10 minutes, cooled and weighed.

Thus, the density, wet weight, dry weight, and ashed weight of each sample was obtained and from these the amounts of water, ash, and organic matter were calculated; the volume was found by dividing the wet weight by the density. Weights of all 3 constituents were expressed in mg. per mm.^{3} of wet tissue and plotted against the density of their respective samples (*figs. 2–5*).

[2] Removal of the excess acid by drying at this point prevents loss of ash by spattering during the final ignition.

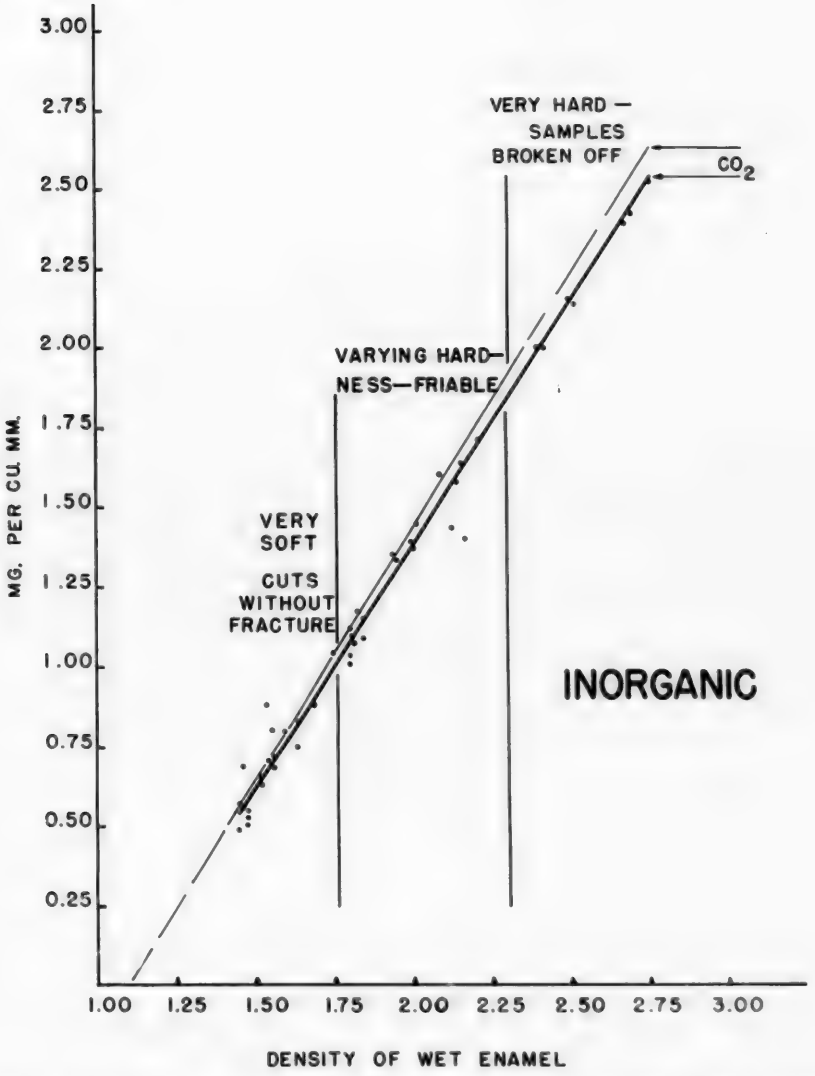

FIG. 2. Inorganic content of developing pig enamel at various densities. The solid line represents the ash content of enamel minus the CO_2 lost in ashing. The broken line represents the total inorganic content, assuming that mature pig enamel (density 2.76) has the same CO_2 content as human enamel, i.e., about 3 to 4 per cent. The upper end of this line is a point 3.5 percent (of 2.76) above the ash line; the lower end is the point found by extrapolating the ash line to zero ash.

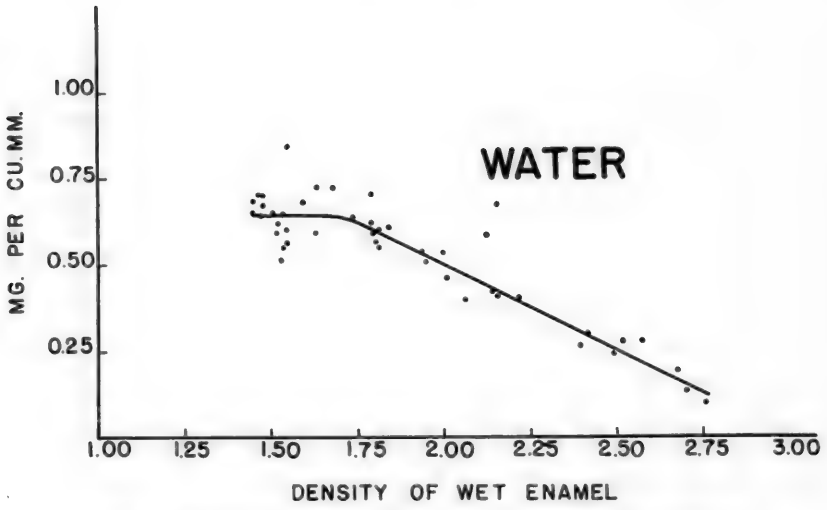

FIG. 3. Water content of developing pig enamel at various densities

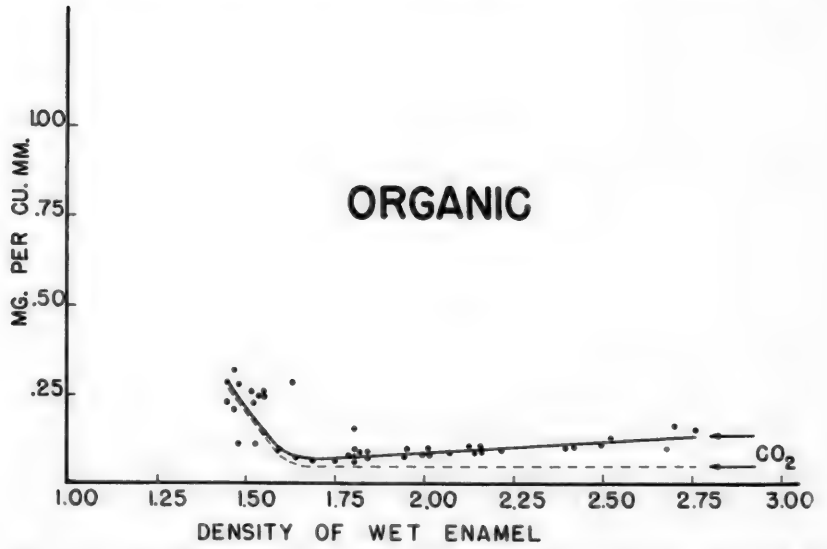

FIG. 4. See *fig. 2* for explanation. The apparent rise in organic content between densities 1.75 and 2.76 is due to loss of CO_2 with the organic matter in ashing. Subtracting CO_2 from the total weight-loss in ashing leaves a constant organic content between these densities (broken line).

RESULTS

The density of enamel from unerupted pigs' teeth varied from 1.45 in the softest samples to 2.70 in the hardest; that of fully formed erupted enamel was

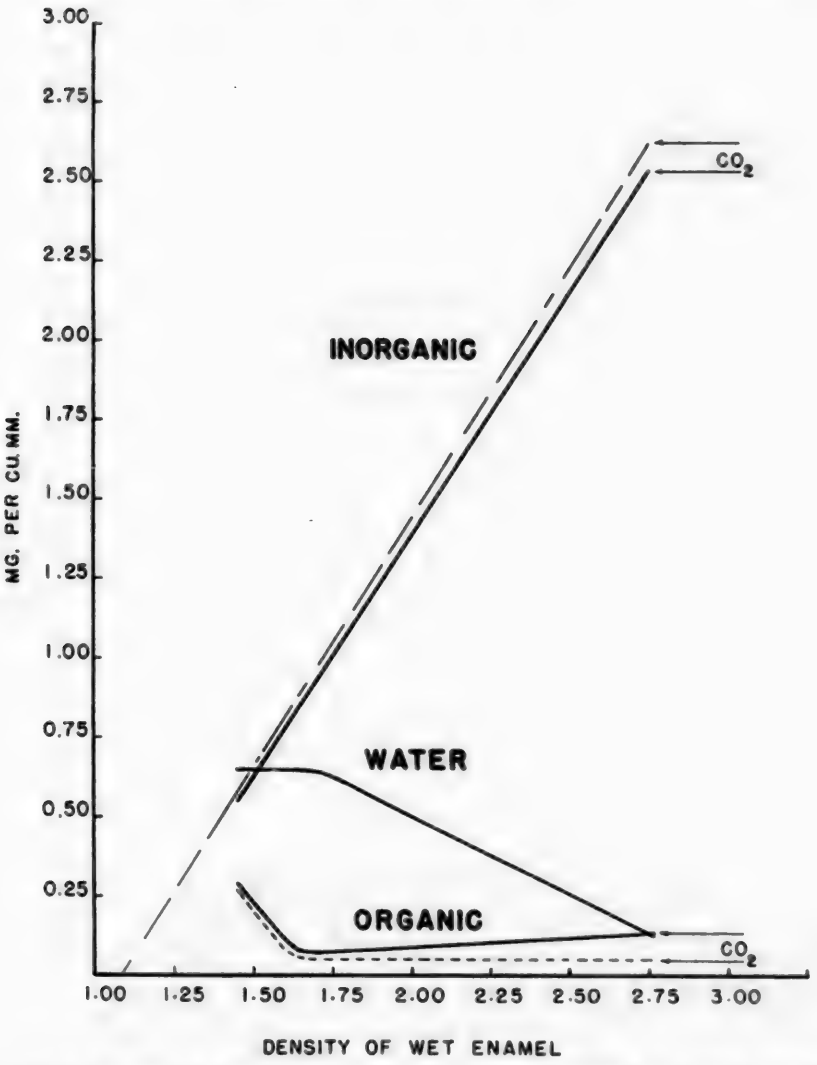

FIG. 5. Summary of chemical changes in developing pig enamel at various densities. The slope of the ash curve is 1.59; the second part of the water curve, -0.5. $\frac{1.59}{3.18} = +0.5$ or slope of a curve which would be the rate of increase in volume of ash per cubic millimeter of enamel. (Weight divided by density is equal to volume.) These two slopes being equal and opposite in sign means that the *volume* of ash deposited just fills the space from which the water was displaced. 3.18 is the accepted density of apatite, the mineral substance in enamel.

2.76. From density 1.45 to 1.75 the samples were very soft and cut easily without fracturing; from 1.75 to 2.25 they were soft enough to cut, but friable;

above density 2.25 all samples were very hard and could not be cut with a scalpel (*fig. 2*).

When the water, inorganic, and organic contents of all samples were plotted against density of the samples, the inorganic content rose from 0.5 to 2.53 mg./mm.^{3} (*fig. 2*). The water content averaged 0.64 mg./mm.^{3} in the samples of lowest density, remained constant from 1.45 to 1.70, and then decreased to 0.12 mg./mm.^{3} in erupted enamel (*fig. 3*). The organic material decreased quickly from 0.27 to 0.05 mg./mm.^{3} between densities 1.45 and 1.65 and remained constant at all higher densities (*fig. 4*).

DISCUSSION

By using density of pig enamel as a criterion of calcification and expressing the chemical composition on a volumetric basis, it has been possible to follow absolute changes in the constituents during the calcification process. Since the

TABLE I

Composition of pig enamel at the lowest and highest degrees of calcification

	LOWEST CALCIFICATION, DENSITY = 1.45, VERY SOFT			HIGHEST CALCIFICATION, DENSITY = 2.76*, VERY HARD			CHANGE IN VOLUME
	mg./mm.^{3}	%	*vol.***	*mg./mm.*^{3}	%	*vol.***	
Inorganic	0.54	37.0	0.16	2.62	95.0	0.82	+0.66
Organic	0.27	19.0	0.20	0.05	1.8	0.04	−0.16
Water	0.64	44.0	0.64	0.12	4.3	0.12	−0.52
	1.45	100.0	1.00	2.79*	101.1	0.98	

* Value 2.76 obtained by flotation; 2.79 is the sum of the weights of the constituents.

** The volume in cu.mm. occupied by each constituent. This was found by assuming densities of 3.18 for inorganic and 1.31 for organic material. The weight divided by density equals volume.

inorganic content increased 5 fold from the softest to the hardest samples, it is evident that the hardening phase of calcification is more than a "crystallization" or "maturation" of inorganic salts already present in the organic matrix.

Quantitatively, this influx of calcium salts is compensated for chiefly by loss of water and, to a lesser extent by loss of organic material (Table I, column 7). Chronologically, the organic material is lost first (after which it becomes constant) and the water later (*fig. 5*). It is important to mention that the increase in hardness occurred only in the period during which water was lost. These events suggest that one phase of enamel hardening is an exchange of inorganic material for water in the organic matrix while the amount of matrix remains constant.

SUMMARY

Forty-five samples of pig enamel at various stages of calcification were analyzed for water, inorganic, and organic matter and the results calculated on a volu-

metric basis to show quantitative changes in each constituent independently of the others.

Apparently there occurs a great influx of ash, *after* the enamel attains its final form, which is compensated for chiefly by loss of water and, to a lesser extent, organic matter. Organic matter is displaced first and water second. The hardening process takes place during the time when water is lost and the organic content remains constant.

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FAILURE TO PRODUCE DENTAL CARIES BY DISTURBING ACID-BASE BALANCE[1]

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In spite of the enormous amount of investigation which it has provoked, dental caries remains today one of the two greatest challenges in the field of dental research. Numerous theories have been advanced, each purporting to hold the solution of the caries problem.

After several years of observation and experimentation on pigs, guinea pigs and rats, Forshufvud (1) reached the following conclusion.

"Caries is ordinarily an expression of disturbed metabolism, often due to inferior diet but certainly more frequently to excessive irregularity in diet and mode of living. No diet can prevent caries when metabolism is disturbed. *Change* in diet, *not only food as such*, causes caries. A *satisfactory and monotonous* diet at regular intervals—without improvised extra meals—is the only one that prevents caries. . . . Extreme caries can be caused, in rats, by variable subcutaneous injections of acid and basic substances."

The same author in another paper (2) stated that an unvarying diet is the best guarantee for good teeth. He used albino rats in experiments designed to alter the acid-base balance by means of daily subcutaneous injections of $NaHCO_3$ or NH_4Cl. When only one of the solutions was injected, 3 rats out of 20 exhibited one carious tooth each. However, when the solutions were alternated daily, caries developed in 12 out of 23 rats in 110 days. These animals were on a diet of whole milk, black bread and carrots. From the experiments mentioned above, Forshufvud concluded: "Decay can be produced experimentally in rats by upsetting acid-base balance without creating local changes in the mouth."

The present study was undertaken in an attempt to duplicate the interesting results which Forshufvud reported.

MATERIALS AND METHODS

Three litters of albino rats, 9, 6 and 9 rats respectively (Table I) were used in this study. One-third of each litter served as controls and received no injections. Of the 16 experimental animals, 6 received daily subcutaneous injections of 3 per cent $NaHCO_3$; the remaining 10 rats were injected alternately with 3 per cent $NaHCO_3$ and 1.5 per cent NH_4Cl for the duration of the experiment. Because the animals were small at the beginning of the study, only ½ cc. of the solutions was injected for each of the first 38 days. All the animals

[1] Presented at the 20th General Meeting of the International Association for Dental Research, March 14-15, 1942, New York, N. Y. (J. D. Res. 21: 338, 1942). Received for publication March 31, 1942.

then weighed over 125 grams. For the next 14 days the daily injection consisted of 1 cc. of solution; then 1½ cc. daily for the following 14 days, and finally 2 cc. daily for the duration of the experiment. Eight (3 control, 5 experimental) of the 11 females bore normal litters during the experimental period (Table I—Miscellaneous Data).

A modified Zucker diet (3) was used, consisting of: ground yellow corn (13%), ground hulled barley (13%), ground whole wheat (13%), ground hulled oats

TABLE I

Data on animals studied in the present investigation

LITTER NUMBER	RAT NO.	AGE AT		LENGTH OF EXP.	NUMBER OF INJECTIONS			BLOOD STUDIES	MISCELLANEOUS DATA
		Start	Sacrifice		NH_4Cl	$NaHCO_3$	Total		
		days	*days*	*days*					
	1	37	176	139	0	0	0		
	2	"	130	93	0	0	0	X	
	3	"	176	139	0	0	0	X	9 young; 127 days
	4	"	176	139	1	111	112		7 young; 112 days
775	5	"	176	139	0	112	112		8 young; 114 days
6♀	6	"	176	139	0	112	112		7 young; 123 days
3♂	7	"	130	93	0	77	77	X	
	8	"	130	93	0	77	77	X	
	9	"	176	139	0	112	112		
	1	32	136	104	0	0	0	X	
	2	"	171	139	0	0	0		7 young; 134 days
801	3	"	171	139	60	54	114		
2♀	4	"	136	104	43	38	81	X	
4♂	5	"	136	104	43	38	81	X	
	6	"	171	139	60	54	114		5 young; 121 days
	1	26	165	139	0	0	0		11 young; 107 days
	2	"	31	5	0	0	0		Accidentally killed
822	3	"	165	139	0	0	0		
3♀	4	"	119	93	41	40	81	X	
6♂	5	"	165	139	54	54	108		
	6	"	119	93	41	40	81	X	
	7	"	31	5	2	3	5		Accidentally killed
	8	"	165	139	54	54	108		6 young; 112 days
	9	"	165	139	54	54	108		

(13%), Soy bean meal (13%), meat scraps (20%), powdered whole milk (10%), alfalfa meal (2%), sodium chloride (not iodized) (2%), calcium carbonate (0.5%). This diet in the form of finely ground particles was fed to the animals while they were small, but later the pellet form was used, in which 0.5 per cent molasses served as the binder. In general, the growth curves of all rats (exclusive of the females during pregnancy) followed those of Zucker.

To determine whether the subcutaneous injections were actually altering the

acid-base balance, cardiapuncture was performed on 9 rats (3 control, 6 experimental) on the final day of the experiment. Blood was collected under paraffin oil not less than 20 minutes or more than 1½ hours after injection of the acid or base solution into the experimental animals, and the Van Slyke microanalysis for CO_2 content of the whole blood was carried out. Dr. Maxwell Karshan, of the Division of Biochemistry, very kindly made the analyses. While definite conclusions cannot be drawn from so few analyses, the results suggest: 1. $NAHCO_3$ raises the CO_2 content of the blood. 2. NH_4Cl lowers the CO_2 content of the blood. (Table II.)

TABLE II

Data concerning blood analyses

LITTER NO.	RAT NO.	CO_2 CONTENT (VOLS. PER CENT)		
		Control	$NaHCO_3$	NH_4Cl
775	3	54.9		
	4		63.4	
	6		64.4	
801	9		59.2	
	2	59.0		
	3			45.0
822	3	49.9		
	5			42.8
	9			50.7
Average		54.6	62.5	46.166

RESULTS

Examination of the jaws and teeth of all rats, by means of the low power binocular dissecting microscope, revealed caries in only one animal. Following are various subjects studied histologically, with our findings.

Dental Caries. In only one animal was there definite advanced caries. This involved the first and second molars in rat 2 of litter 775 (*fig.* 1A, B). From Table I it will be seen that this animal was a control, received no injections and was 130 days old at sacrifice. Rat 5 of litter 775 had an early carious lesion of the enamel matrix of the lower right first molar (*fig.* 2). Table I shows that this animal received 112 injections of $NaHCO_3$ during the 139 days of the experiment, and was 176 days old at sacrifice. This is a very early lesion. It is important to point out that Forshufvud claims the "monotonous" diet prevents caries, and that he found the least amount of caries when he injected the same solution day after day, as was done in this animal.

The only other animal which showed any evidence of dental caries was rat 4 of litter 822. Table I shows that this animal received 41 injections of NH_4Cl and 40 injections of $NaHCO_3$ for a total of 81 injections during the 93 days ex-

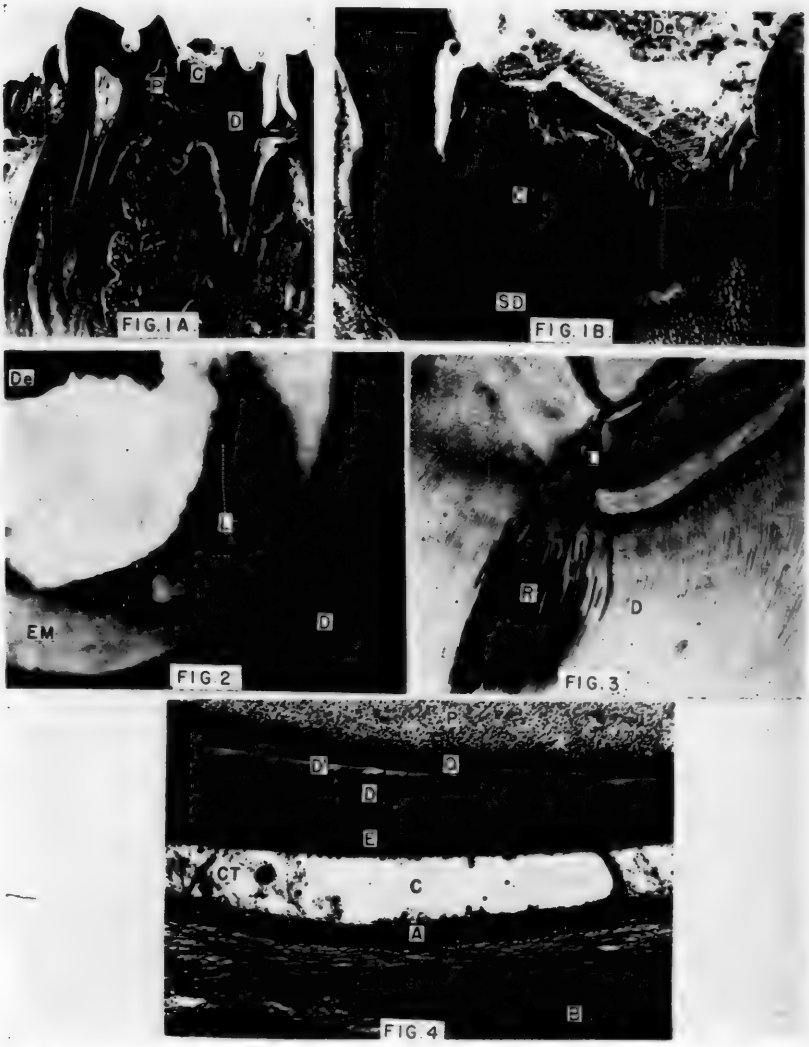

FIG. 1. Dental caries, right mandibular first molar. Decalcified section. Control animal. Rat 2, litter 775. (See Table I.) A. Note destruction of dentin in groove (C), and large amount of secondary dentin under carious lesion. Orig. mag. 29 ×. B. Higher magnification of area C from *fig.* 1A. Orig. mag. 400 ×. P—dental pulp, D—dentin, SD—secondary dentin, C—carious lesion, De—debris.

FIG. 2. Early carious lesion of enamel matrix, right mandibular first molar. Rat 5, litter 775. Decalcified section. Orig. mag. 1000 ×. EM—enamel matrix, D—dentin, De—debris in occlusal groove, L—lesion of enamel matrix.

FIG. 3. Early enamel lesion with reaction in dentin. Left mandibular first molar. Rat 4, litter 822. Ground section. Orig. mag. 400 ×. E—enamel, L—enamel lesion, D—dentin, R—reaction area in dentin.

FIG. 4. Area from right mandibular incisor. Rat 7, litter 775. Abnormal space (c) between enamel surface and ameloblast layer contains shreds of tissue (ct). Note the many calcific globules of various sizes, on the surface of the enamel and in the meshes of the abnormal tissue. Decalcified section. Orig. mag. 175 ×. P—dental pulp, O—odontoblast layer, D'pre-dentin, D—dentin, E—enamel, A—ameloblast layer, B—bone.

perimental period. This is a very early lesion; whether it is a fracture lesion originating in the groove, or true caries, is somewhat debatable (*fig.* 3).

Secondary dentin. The occurrence of secondary dentin was seen in the first and second molars under areas of attrition. The only 2 animals not showing secondary dentin died at 31 days, before secondary dentin developed.

Pulp nodules. These calcified bodies are of uncommon occurrence in the pulp tissues of the albino rat (especially in the age group included in this experiment). Only one animal, 3 of litter 801 developed such a concretion, in the radicular pulp of the left maxillary first molar. The main body of the mass consisted of a cellular material which stained a light pink with eosin, very similarly to predentin. It was surrounded by cells which resembled atrophic odontoblasts. Although the finding of this one pulp nodule is interesting, no significance can be

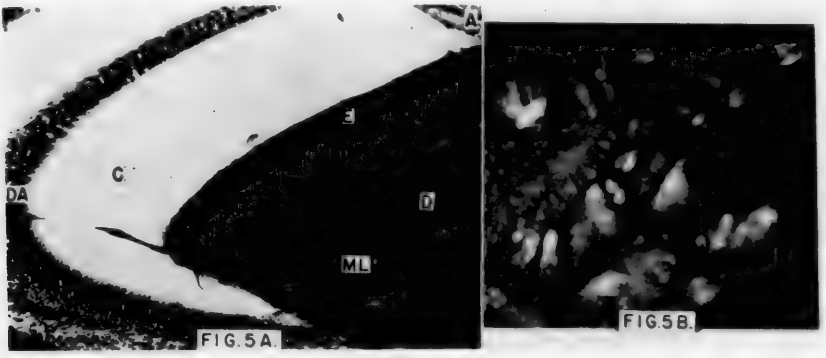

FIG. 5. Enamel matrix lesion, incisor. Rat 8, litter 822. (See Table I). Decalcified section. A. Note cyst-like space (c) around formative end of incisor; also note disturbance of enamel matrix in area ML. Orig. mag. 175 ×. D—dentin, E—enamel, A—ameloblastic layer, DA—degenerated ameloblast layer around space C. B. Higher magnification of area ML from 5 A. Orig. mag. 400 ×. Note how direction of enamel rods is changed and their pattern altered in the abnormal area.

attached to the fact that it was present in an animal receiving alternate injections of NH_4Cl and $NaHCO_3$.

Odontoblasts. Except for an occasional area under a thick deposit of secondary dentin in the first or second molars, the odontoblasts appeared normal in all cases. Under the heavy layers of secondary dentin the odontoblasts appeared atrophic, or were absent over small areas. This was also true under the advanced carious lesion described above. It is our impression that the odontoblasts were not affected by the experimental procedures performed on these animals.

Ameloblasts. The ameloblastic layer was studied in each of 24 rats, in the incisor teeth, and also in the molars in those cases where the enamel of the molar crowns was not completely calcified. In 6 animals there was a disturbance of the ameloblasts due to an accumulation of fluid between the last formed enamel matrix and the ameloblast layer. This caused the appearance of pressure

atrophy of the ameloblasts lining the fluid-filled cavity. This condition was found in a molar tooth of rat 5 of litter 822. Each of the other 5 rats show a cyst-like space between the surface of the enamel matrix of the incisor tooth and the adjacent ameloblasts. The size of the area varies in each of the 5 animals. *Fig.* 4 illustrates this condition in rat 7 of litter 775. It is interesting to notice the abnormal network of tissue which exists between the ameloblasts and the surface of the enamel matrix. The fact that some of the ameloblasts continued their activity after the beginning of the disturbance is evidenced by the presence of rounded globules between the ameloblasts and the surface of the formed enamel matrix. These globules show staining reactions identical with those of the enamel matrix. While we are not prepared to explain the reason for the cyst-like areas in the ameloblast region of the incisors, there can be little doubt that they are pathological. The animals showing the condition are 7 of litter 775 and 1, 3, 4, 5, 8 of litter 822. Table I shows that 2 of these were control animals; 1 received only $NaHCO_3$; and the other 3 received alternate injections of $NaHCO_3$ and NH_4Cl. From such distribution we must conclude that the disturbance is not due to the injection of the acid or base solutions.

Enamel Matrix. In one case, mentioned under the discussion on caries, there was an early carious lesion of the enamel matrix of a molar (*fig.* 2). In only one other animal was a disturbance of enamel matrix observed and this was in the incisor of rat 8 in litter 822. This is seen in *fig.* 5A which also shows the cyst-like cavity discussed under ameloblasts. *Fig.* 5B shows the area of affected enamel matrix under higher enlargement. The enamel rods appear irregular and indistinct in the affected area. What the etiology of this lesion is, we cannot say, but since it was seen in only one animal, we do not feel it can be ascribed to the acid or base solutions used in the experiment.

SUMMARY

Daily subcutaneous injections of $NaHCO_3$ (6 animals), or alternate daily injections of $NaHCO_3$ and NH_4Cl (9 animals) failed to produce dental caries in albino rats. Histologic examination of the rats' teeth showed occasional abnormalities of the enamel matrix, ameloblasts, and odontoblasts. It was not possible to associate any of these conditions with the injection of either of the solutions used in the experiment.

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THE MEASUREMENT AND COMPARISON OF DENTAL CARIES EXPERIENCE[1]

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During the past decade there have been notable changes in the measurement of dental caries experience. Frequently the percent of the population observed to have carious teeth was recorded. When it was desired to compare 2 populations with respect to the presence of caries the percent of each population with 1 or more carious teeth was determined and the 2 calculated percentages were compared. In such comparisons much is lost since each member of either population is considered only as having or not having dental caries, no account being taken of the degree or intensity of affection associated with a carious mouth. Thus the person with, say, 3 carious teeth is not differentiated from the person with 1 carious tooth.

Subsequently attention was directed to the degree of affection. Reference is made to the investigations of the U. S. Public Health Service conducted by Collins, Gafafer, Dean, Palmer, Klein, and their coworkers. The reports on these investigations have appeared principally in the U. S. *Public Health Reports*, and in some instances as U. S. *Public Health Bulletins* (for example, 1–6). It is unnecessary to present further historical details since these are readily available in the various publications by the investigators to whom reference has been made.

With respect to the published work pertaining to degree of affection the analyses deal primarily with the number of teeth found decayed, missing or filled in each mouth examined. A quantitative expression of the caries experience for each mouth is obtained by summing the numbers of permanent teeth associated, respectively, with the 3 classes: decayed, missing and filled. The various summations, one for each mouth examined, may be totaled, divided by the number of persons examined, and multiplied by 100. This procedure yields for the population examined the number of decayed, missing and filled permanent teeth per 100 persons, a rate which may be reasonably defined as a measure of the caries experience of a population.[2] When such a rate is calculated it is desirable to compare it with the corresponding rate found for another population. Difficulties are encountered if it is attempted to compare these rates after the manner employed

[1] Presented at the 20th General Meeting of the International Association for Dental Research, New York, March 14 and 15, 1942. (*J. D. Res.* **21**: 333, 1942.)

[2] The rate as defined has been usually determined for school children. When adults are examined, particularly those 30 or 35 years of age and over, the rate is less likely to reflect caries experience since the component of the rate determined by the number of missing teeth is more likely to reflect causes other than caries.

in connection with the type of rate earlier referred to, namely, the percent of a population observed to have 1 or more carious teeth. It is purposed in this paper to differentiate between the 2 types of rate and to provide for each an answer to the question: What is the probability that the 2 rates differ as much as they do by the operation of chance alone?

RATIOS: RATES AND FREQUENCY RATES

When a number, a, is related to another number, b, symbolized by the quotient, a/b, the ratio of a to b results. There is no restriction on the size of a in relation to b; moreover, if a and b represent measurements there is no restriction whatever with regard to the kind of unit in which a or b is measured. Since b may have a value of 1, all numbers are ratios. As another example may be cited the ratio, for a particular community, of the population to the number of practicing dentists.

Any ratio can be expressed as a rate if the indicated division is carried out, and an appropriate multiplication is performed to express the new b as a multiple of 10. Thus the caries experience rate (number of decayed, missing and filled permanent teeth per 100 persons) is derived from the ratio, number of decayed, missing and filled permanent teeth to the number of persons examined, and multiplied by 100.

Any ratio a/b with the a and b units the same, and a a part of b, or equal to b, gives the *frequency* of occurrence, a, of a particular attribute among a population of b persons or objects. It will be observed that the a represents the number of persons or objects with the particular attribute, and b the number with and without the attribute. When the frequency of occurrence is related to a population base expressed as a multiple of 10, say 100, or 1,000, objects or persons, a *frequency rate* results. When the base is 100 the frequency rate is equivalent to a percentage; on the other hand when the base is 1 which is obtained by dividing the frequency a by the population b, the quotient is a *probability* which indicates the probability of the occurrence of the particular attribute in a population similar to the given one. Thus if 180 persons in a population of 200 persons show one or more carious teeth, the frequency is 180 while the frequency rate is 90 per 100; moreover, the percentage of persons affected in this population is 90, and the probability of a person's being found with carious teeth in a similar population is 0.9. Again, of 1,000 cities, 200 cities may show among children, a caries experience rate of 300. Thus the attribute, 300 decayed, missing and filled permanent teeth per 100 children, occurs 200 times in this group of 1,000 cities. The frequency rate, therefore, is 20 per 100 and the percentage is 20 percent; the probability of occurrence of a city with a caries experience rate of 300 in a similar group of cities is 0.2.

The caries experience rate, determined by the summation of the decayed, missing and filled permanent teeth in a population and related to the number of persons in the population, is not a frequency rate and hence cannot be expressed as a percentage or a probability. The rate would be a frequency rate if the b of the ratio were the entire possible tooth population, that is, the number of persons

examined multiplied by 32 (or 28), a situation which would yield a certain number of decayed, missing and filled teeth per 100 teeth, as well as a percentage, and a probability.

Because the caries experience rate as defined is not a frequency rate difficulties are introduced when such rates are compared after the manner employed with respect to the rate denoting the percent of a population observed to have one or more carious teeth.

It should be stated that all rates involving events that may affect a person or object more than once present a similar situation with regard to rate comparisons. The sickness rate is an example since it involves multiple attacks (7). If a person were naturally limited to 32 (or 28) sicknesses during his lifetime, and past sicknesses were recorded, the example would be particularly appropriate on this occasion.

Rates of change: It is desirable to call attention to the fact that the illustrative examples presented above had to do entirely with static conditions. Thus the caries experience rate, as defined, denotes the experience of a particular group at a particular time. The same holds for the frequency rate. The dynamic element may be introduced into any ratio (which includes rates and frequency rates) by ascertaining the change in the ratio with respect to, for example, time or space. Thus a group of children may be observed annually over a number of years and as a result there may be determined for a chosen age interval the change in the caries experience. This change is expressed as a rate, namely, a certain number of decayed, missing and filled permanent teeth per 100 children per year of life and represents an average rate of change in caries experience with respect to age.

COMPARISON OF MEASURES OF CARIES EXPERIENCE[3]

Frequency rates or their corresponding percentages: A. The results (2) of a dental examination of 558 white and 130 colored males, 16–20 years of age, may be summarized as shown in the accompanying fourfold table. It will be observed that in the total population of 688 white and colored males, 582 or 84.59 percent (a

CARIES	AGES, 16–20 YEARS		TOTAL
	White	Colored	
	(a)	(b)	(a + b)
Present	481	101	582
	(c)	(d)	(c + d)
Absent	77	29	106
	(a + c)	(b + d)	(N or a + b + c + d)
Total	558	130	688

frequency of 84.59 males per 100 males) showed evidence of past or present caries. The corresponding percentages for the white and colored males, yielded by 481/558 and 101/130, are 86.20 and 77.69, respectively. Are the percentages 86.20 and 77.69 significantly different, or is the difference due to chance? Are white males, 16 to 20 years of age, more likely to have signs of caries than colored

[3] Compare Gafafer (8).

males of the corresponding age group? A repetition of the investigation using samples of like sizes would probably yield different percentages; in fact the percentages might be reversed. Is the difference 86.20 minus 77.69, or 8.51 percent sufficiently large to make the investigator feel that it is real? How probable is it that 2 random samples of 558 and 130, respectively, drawn from a universe with a basic percentage of past or present caries of 84.59, will show a difference as large as or larger than the size found in the present experience? To answer this question it is necessary to know that the variability of a percentage expected from random sampling alone is given by *sigma*, thus

$$\sigma = \text{standard error} = \sqrt{pq/n}$$

where p = percentage of the universe
q = 100 − p, and
n = size of the sample.

The standard errors of the 2 samples are, therefore,

$$\sigma_{white} = \sigma_w = \sqrt{(84.59)(15.41)/558} = 1.53 \text{ percent},$$

$$\sigma_{colored} = \sigma_c = \sqrt{(84.59)(15.41)/130} = 3.17 \text{ percent}.$$

The standard error of the difference between the observed percentages is

$$\sigma_{diff.} = \sqrt{\sigma_w^2 + \sigma_c^2} = \sqrt{(1.53)^2 + (3.17)^2} = 3.52 \text{ percent}.$$

The observed difference between the 2 colors is 8.51 percent, and in terms of the standard error of the difference, 3.52 percent, it is 8.51/3.52 or 2.4. Since a deviation of 2 or more sigmas is generally accepted as being unlikely to be due to chance, the investigator feels that the present experience limited to males 16 to 20 years old, indicates a race differential,[4] and that if the samples are representative, white males show a greater tendency to be attacked by caries than colored males. With regard to the probability of occurrence in 100 trials of a statistical deviation of 2.4 or more sigmas, a table in Pearl (9, p. 473) shows it to be 1.6. In other words, a deviation of 2.4 or more sigmas occurs by chance only 1.6 times in 100 trials.

B. Another procedure for comparing frequency rates or their corresponding percentages is afforded by the chi-square test which requires the calculation of chi-square for a fourfold table. The value of chi-square for a fourfold table is given by the following,

$$x = \frac{(ad - bc)^2 N}{(a + b)(c + d)(a + c)(b + d)},$$

the letters a, b, c, d and N corresponding to those shown in the fourfold table presented above. When the absolute frequencies shown in the table are substituted in the above expression, the following results:

$$x^2 = \frac{[(481)(29) - (77)(101)]^2\, 688}{(582)(106)(558)(130)} = 5.86.$$

[4] This finding is in harmony with current knowledge.

Since a chi-square of 4.0 or more *yielded by a fourfold table* is generally accepted as pointing to a significant difference between the percentages compared, the present experience indicates a race differential or an association between caries experience and race, the white males being more likely to suffer from this malady than the colored ones. With respect to the probability involved the determined chi-square value of 5.86 may be converted into a probability, P, with the use of the graph presented as *fig. 1*. For an n′ of 2 (the number of attributes or paired frequencies or the number of rows in the fourfold table) the value of P is seen to be less than .02 but greater than .01.[5] This value of P means that in only 1.5 (approximately) out of 100 trials would there be a sampling difference between the races as large as or larger than the one observed. The observed difference, therefore, is unlikely to have occurred by chance.

Rates involving multiple events: Reference has been made to the fact that the caries experience rate defined by the number of decayed, missing and filled permanent teeth per 100 persons is one that involves multiple events. Since the rate is not a percentage the procedures presented in the previous section are not directly applicable to making a comparison of 2 rates of this type. However since a rate involving multiple events is derivable from a frequency distribution, that is, a set of frequencies, the comparison of 2 rates of this type may be performed by comparing the corresponding frequencies of the 2 frequency distributions with the use of principles applicable to frequency rates.

The subject may be clarified with an example based on dental caries experience data on 12 to 14 year old children in 3 cities, X, Y, and H. City X is represented by the combined experience of several communities as reported by Dean (4), City Y represents one community likewise reported upon by Dean (3), and the experience of City H is hypothetical. The pertinent data are presented in Table I. It will be observed that the dental caries experience rates for the 3 cities X, Y and H, are 746, 633 and 583, respectively, the percentage of children found caries-free being 3.7, 3.9 and 4.7. The 3 frequency distributions are presented graphically in *fig. 2*.

Two questions are proposed. First, is the experience rate of City X significantly different from the experience rate of City Y? And, second, is the experience rate of City Y significantly different from the experience rate of City H? Since the 3 rates involve multiple events the frequency distributions yielding the rates, rather than the rates themselves, are compared. The frequency distributions are shown in columns 1, 2 and 3 of Table I. To avoid ambiguity let the immediate discussion be concerned with the first question. This question has to do with the caries experience rates of Cities X and Y, and the 2 frequency distributions may be compared, first, by forming a fourfold table with the use of an "appropriate split" in the number of attacks suffered followed by the application of the chi-square test of the last section, or, second, by an application of a chi-

[5] A more precise value of P, namely, .01550, may be obtained by interpolation from a table, for example, Yule and Kendall (10, pp. 534–535). This table gives the different P values corresponding to various values of chi-square, the n′ being limited to the value, 2.

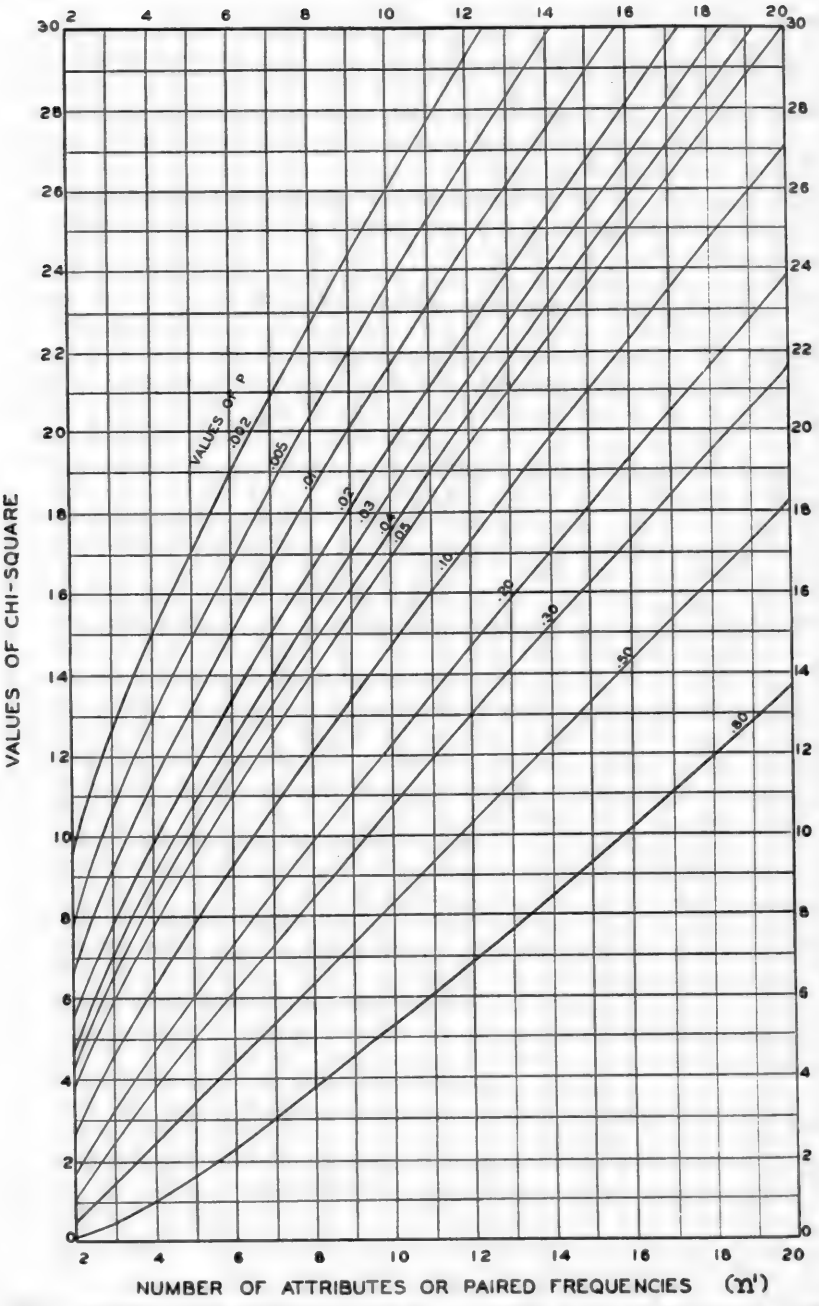

FIG. 1. Relation of P to chi-square and n'. A particular value of chi-square as determined by procedure outlined in text and particular value of n' yield probability, P, that 2 distributions are random samples from same population.

TABLE I

Distribution of the occurrence of multiple attacks of dental caries among 12 to 14 year old children of 3 cities

ATTRIBUTE, OR NUMBER OF ATTACKS DECAYED, MISSING AND FILLED PERMANENT TEETH)	FREQUENCY DISTRIBUTION, OR NUMBER OF CHILDREN WITH INDICATED ATTRIBUTE			PERCENTAGE FREQUENCY DISTRIBUTION		
	City X [From Dean (4)]	City Y [From Dean (3)]	City H [Hypothetical]	City X	City Y	City H
A	(1)	(2)	(3)	(4)	(5)	(6)
None, or one or more	1,008	306	319	100.00	100.00	100.00
0	37	12	15	3.67	3.92	4.70
1	27	18	22	2.68	5.88	6.90
2	46	16	20	4.56	5.23	6.27
3	57	27	23	5.65	8.82	7.21
4	113	36	34	11.21	11.77	10.66
5	94	34	39	9.33	11.11	12.23
6	95	38	30	9.42	12.42	9.40
7	84	22	35	8.33	7.19	10.97
8	101	33	41	10.02	10.78	12.85
9	68	21	20	6.75	6.86	6.27
10	67	11	15	6.65	3.60	4.70
11	49	7	10	4.86	2.29	3.14
12	40	6	7	3.97	1.96	2.19
13	42	5	4	4.17	1.63	1.26
14	15	5	0	1.49	1.64	0
15	15	3	2	1.49	.98	.63
16	13	3	0	1.29	.98	0
17	13	1	1	1.29	.33	.31
18	11	5	0	1.09	1.63	0
19	6	1	0	.59	.33	0
20 or more	15	2	1	1.49	.65	.31
Number of decayed, missing and filled permanent teeth per 100 children	746	633	583	—	—	—

square test covering all of the corresponding frequencies of the 2 frequency distributions. With regard to an "appropriate split" the following fourfold table shows the results of making one of a number of possible splits:

NUMBER OF ATTACKS	CITY X	CITY Y	TOTAL
Less than 4	167	73	240
4 or more	841	233	1,074
Total	1,008	306	1,314

The second chi-square test referred to is the more severe since the entire frequency distributions are involved in the comparisons. The question of whether

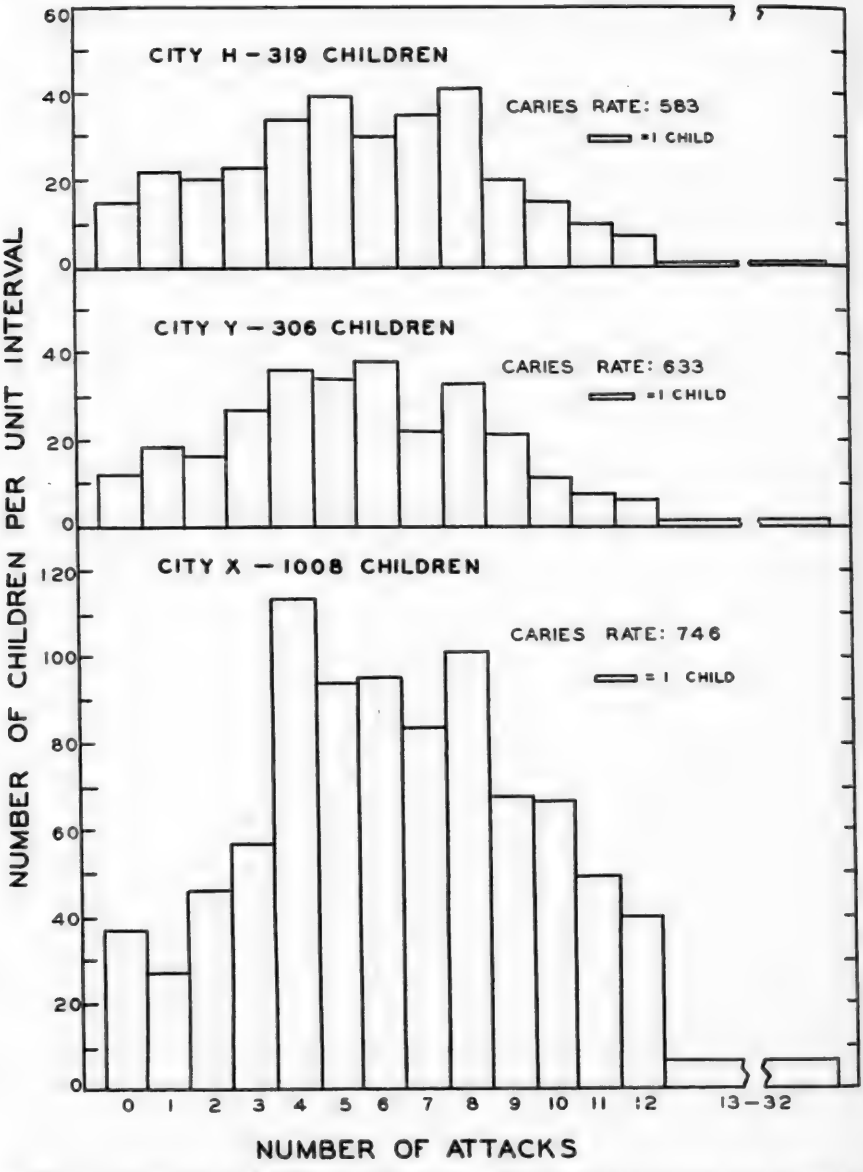
CITY H – 319 CHILDREN
CARIES RATE: 583
= 1 CHILD
CITY Y – 306 CHILDREN
CARIES RATE: 633
= 1 CHILD
CITY X – 1008 CHILDREN
CARIES RATE: 746
= 1 CHILD
NUMBER OF CHILDREN PER UNIT INTERVAL
0 20 40 60
0 20 40
0 20 40 60 80 100 120
0 1 2 3 4 5 6 7 8 9 10 11 12 13–32
NUMBER OF ATTACKS

FIG. 2. Histograms for 3 different cities showing distribution of children with respect to multiplicity of attacks of dental caries. *Note:* For illustrative purposes it is not entirely objectionable to use 32 as the maximum number of decayed, missing and filled permanent teeth. However, in epidemiological studies it is desirable to limit tooth population of mouth to 28 teeth, excluding third molars.

the 2 caries experience rates (746 and 633) are significantly different may be stated specifically in terms of the 2 distributions yielding the 2 respective rates,

thus: Do the 2 distributions with respect to the frequency of occurrence of multiple attacks of caries differ from each other only by so much as might arise by chance in repeated sampling? Or, what is the probability that the 2 distribu-

TABLE II

*Distribution of the occurrence of multiple attacks of dental caries among 12 to 14 year old children of 2 cities; calculation of chi-square to determine the significance of the difference between the caries experience rates**

ATTRIBUTE, OR NUMBER OF ATTACKS (DECAYED, MISSING AND FILLED PERMANENT TEETH)	FREQUENCY DISTRIBUTION, OR NUMBER OF CHILDREN WITH INDICATED ATTRIBUTE		$F + f$ (1) + (2)	F/N	f/n	F/N − f/n (4) − (5)	(F/N − f/n)² (6) SQUARED	$\frac{(F/N - f/n)^2}{F + f}$ (7) ÷ (3)
	City X F	City Y f						
A	(1)	(2)	(3)	(4)**	(5)	(6)	(7)	(8)
0	37	12	49	.0367	.0392	−.0025	.00000625	.00000013
1	27	18	45	.0268	.0588	−.0320	.00102400	.00002276
2	46	16	62	.0456	.0523	−.0067	.00004489	.00000072
3	57	27	84	.0565	.0882	−.0317	.00100489	.00001196
4	113	36	149	.1121	.1177	−.0056	.00003136	.00000021
5	94	34	128	.0933	.1111	−.0178	.00031684	.00000248
6	95	38	133	.0942	.1242	−.0300	.00090000	.00000677
7	84	22	106	.0833	.0719	.0114	.00012996	.00000123
8	101	33	134	.1002	.1078	−.0076	.00005776	.00000043
9	68	21	89	.0675	.0686	−.0011	.00000121	.00000001
10	67	11	78	.0665	.0360	.0305	.00093025	.00001193
11	49	7	56	.0486	.0229	.0257	.00066049	.00001179
12	40	6	46	.0397	.0196	.0201	.00040401	.00000878
13	42	5	47	.0417	.0163	.0254	.00064516	.00001373
14	15	5	20	.0149	.0164	−.0015	.00000225	.00000011
15 or more	73	15	88	.0724	.0490	.0234	.00054756	.00000622
Σ or Sum	1,008 or N	306 or n	1,314	1.0000	1.0000	.0000	—	.00009926

* The caries experience rates (number of decayed, missing and filled permanent teeth per 100 children) for cities X and Y are 746 and 633, respectively.

** This column is a set of probabilities. The probability of a person's being attacked 0 times is .0367, once is .0268, ····, and 15 times or more is .0724. Column 5 is likewise a set of probabilities. For a population similar to that of City X (or City Y) the number of persons expected to suffer a given number of attacks may be obtained by multiplying the number of persons in the population by the appropriate probability given in column 4 (or column 5).

$$\chi^2 = Nn \sum_{A=0}^{A=15+} \frac{(F/N - f/n)^2}{F + f} = (1{,}008)(306)(.00009926) = 30.61654848$$

Since $n' = 16$, $P = .01$. Therefore the 2 rates are significantly different.

tions are merely random samples of the same population? The value of chi-square is a determining factor of this probability, P.

Table II shows the frequency distributions, F and f, and the arithmetic procedure connected with the computation of chi-square. It will be observed that the right-hand tail of each distribution has been summed beyond the Attribute

"14" since it is undesirable to have a frequency of less than 5 entering the test. It is seen (Table I) that the distribution for City Y has frequencies of less than 5 for the Attribute 15 and higher. These frequencies are therefore summed. The distribution for City X is likewise treated since the test requires that each Attribute be represented by a frequency from each distribution.

A further inspection of Table II reveals that chi-square is determined by a product involving 3 terms, namely, the number of children (N) yielding the first distribution, the number of children (n) yielding the second distribution, and a certain summation. The summation is determined by a contribution from each row or line of the table, in other words, from each Attribute (A). It will be observed that the greater the differences between the distributions as measured by differences in corresponding probabilities ($F/N - f/n$), the larger will chi-square be. On the other hand, if the 2 distributions are precisely the same, the chi-square is zero. Hence a sufficiently large chi-square value is associated with 2 distributions that are significantly different while a sufficiently small chi-square value is characteristic of 2 distributions that are not significantly different.

With a summation extending over the range, Attribute = 0 to Attribute = 15 or more, the chi-square is seen to be 30.6. There are many probability values (P) corresponding to this value of chi-square, the particular value depending upon the size of n' which is the number of pairs of Attributes entering the test or the number of rows in the table used in computing chi-square. In the present instance n' is 16. According to the graph presented as *fig. 1* the P corresponding to a chi-square of 30.6 with $n' = 16$ may be seen to be less than .01, an extension of the 2 lines $n' = 16$ and $P = .01$ being necessary to facilitate the determination.[6] Since a P of .04 or less is generally considered as indicating that 2 distributions are not random samples from the same population, the 2 distributions under consideration are felt to be significantly different, and hence the 2 cities, X and Y, present different caries experience rates. This finding logically permits the seeking of an hypothesis to explain the observed difference.

Attention is now directed to the frequency distributions for cities Y and H (Table I). The chi-square value in this instance is found to be 16.4. With a value of $n' = 14$ (the right-hand tail of each distribution having been summed for 13 or more attacks), the P, according to *fig. 1*, is .25, meaning that a difference as large as observed or larger would be found 25 times out of 100 upon repeated sampling. This probability (or any probability larger than .04) is considered sufficiently large to indicate that the 2 distributions are random samples from the same population. Hence the difference between the 2 distributions is likely to be due to chance. Thus in this hypothetical situation the evidence indicates that there is unlikely to be a difference between the 2 cities, H and Y, with respect to caries experience.

Effect on value of chi-square of changing sizes of populations examined: It is of interest to determine the effect on chi-square of changing the sizes of the 2 populations entering the

[6] More precise values of P may be found in a table in Pearson (11, pp. 26–28). This table shows values of chi-square and n' in the stub and head, respectively, and values of P in the body of the table. The values of n' cover the range, 3 through 30.

test. Let it be assumed that each frequency shown in columns 1 and 2 of Table II be divided by 3. As a consequence the examined populations of sizes, N and n, become N/3 and n/3, respectively; each (F + f) in column 3 becomes (F + f)/3; and columns 4, 5, 6 and 7 remain the same. Column 8 has each of its items multiplied by 3 since (F + f) has become (F + f)/3, and the summation for this column thus becomes 3 times its previous value. The new value for chi-square, therefore, is (N/3)(n/3)(.00009926)(3) or one-third of its previous value. In general when each frequency of 2 frequency distributions entering a chi-square test is divided or multiplied by some value, V, chi-square is divided or multiplied by V. Since P varies inversely with chi-square, population reductions of this kind make the difference between 2 rates relatively less significant, while increases make the difference relatively more significant. It should be observed that these changes in population do not affect the magnitudes of the caries rates since the number of decayed, missing and filled teeth as well as the population examined is multiplied or divided by the same number.

Finally, as would be expected, multiplying or dividing by V the frequencies in a fourfold table multiplies or divides by V the value of chi-square derived from the table.

SUMMARY

Dental caries experience rates representing probabilities may be directly compared with the use of ordinary methods. The dental caries experience rate, number of decayed, missing and filled permanent teeth per 100 persons examined, on the other hand, involves multiple events. To compare rates of this kind requires knowledge of the frequency distributions yielding the rates. A procedure of comparison involving the chi-square test was presented.

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TREND IN ATTACK RATE OF DENTAL CARIES OF 358 UNITED STATES COUNTIES[1]

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Dental caries in our opinion should be considered a pathological condition affecting the dental apparatus as a unit. We have suggested that this apparatus be termed the *Biodenture*. Elsewhere, we stated that statistics of dental caries should be so collected and arranged that it is possible to determine the number of children who are free from caries, and those who have one or more DMF (decayed, missing or filled) teeth. This arrangement permits a Health Index of the teeth (1).

In another paper we suggested that dental statistics should permit a more complete understanding of the distribution of caries in children than would be afforded by the simple Health Index of the teeth. We suggested that the different ratios of children with none, 2 and so forth DMF teeth be given (2).

The ratio of the number of DMF teeth per 100 children is not entirely satisfactory, because it does not take into account the increasing number of teeth in the mouths of children as they become older. For example, "relation of DMF teeth per 100 children," if compiled for the various ages of childhood, compares children of seven, having an average of but 10 permanent teeth and children of 14 probably having 28 permanent teeth.[3] If the number of DMF teeth was related to the total number of teeth in the mouths of the observed children, the relationship would be a more proper one. However, even this ratio would not be entirely satisfactory.

The following will demonstrate the difficulty. A child of 10 will probably have 18 permanent teeth. Four of the 18 will be first molars. The first molars will have been in the mouth, on the average, 4½ years. Two of the 18 permanent teeth will be the lower canines. They will have been in the mouth 1½ years.[4] The remaining 12 teeth will have been exposed in the oral cavity for different amounts of time corresponding to their various eruption dates. The amount of time which has elapsed between the eruption and examination of a tooth can be said to be the exposure time. The sum of the various exposure times of the individual teeth gives the total exposure time for a given age at a particular date

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[3] In this paper we deal only with permanent teeth. Hereafter when teeth are mentioned it will be understood that we are referring to permanent teeth.

[4] We consider a child to be of a certain age until he has reached the next birthday.

of examination. From these data, a proper ratio is obtained when the DMF prevalence is related to the total number of teeth and the accumulated time the teeth have been in the mouth. We depended on the eruption tables of Kronfeld to determine the exposure time of the different teeth (3). We realize that the eruption time-tables are only approximate but for our purposes they seem to be satisfactory until more accurate information is available.[5] In Table I we give the exposure units for the various permanent teeth at different ages.

In the study on prevalence of caries and post-eruptive tooth age in children of Bergen County, New Jersey, we found a striking regularity of caries increase with increasing exposure time of teeth. We found that the values for the number of DMF teeth per 100 children for the different exposure time units, plotted

TABLE I

Accumulated exposure units for permanent teeth

Age	6	7	8	9	10	11	12	13	14	15	16	17
	Accumulated Exposure Units of Permanent Teeth (Post-Eruptive Tooth-Age in Years) →											
Lower 1st Molars	1	3	5	7	9	11	13	15	17	19	21	23
Upper 1st Molars	1	3	5	7	9	11	13	15	17	19	21	23
Lower 1st Incisors	1	3	5	7	9	11	13	15	17	19	21	23
Upper 1st Incisors		1	3	5	7	9	11	13	15	17	19	21
Lower 2nd Incisors		1	3	5	7	9	11	13	15	17	19	21
Upper 2nd Incisors			1	3	5	7	9	11	13	15	17	19
Lower Canine				1	3	5	7	9	11	13	15	17
Upper 1st Premolars					1	3	5	7	9	11	13	15
Lower 1st Premolars					1	3	5	7	9	11	13	15
Upper 2nd Premolars						1	3	5	7	9	11	13
Lower 2nd Premolars						1	3	5	7	9	11	13
Upper Canines						1	3	5	7	9	11	13
Upper 2nd Molars							1	3	5	7	9	11
Lower 2nd Molars							1	3	5	7	9	11
Total for Biodenture	3	11	22	35	51	72	98	126	154	182	210	238

on regular graph paper, followed, very closely, parabolic curves. When these values were plotted according to the logarithmic system, very accurate straight lines resulted. The question now arises whether that striking regularity is peculiar to the Bergen County data or is it a demonstration of a major rule following a hidden biological law of dental decay. We do not refer to the almost universal incidence and prevalence of caries in civilized children but only to the regularity of the trend of increase. For the purpose of testing that possibility, we have used part of the extensive data collected under the direction of and

[5] More extensive investigations have been made of the mean eruption of teeth. An example would be that of Klein and Palmer of the Hagerstown, Maryland, children. However, these accurate findings might not apply to the teeth of children residing in areas having more or less favorable environmental conditions.

published by, the United States Public Health Service (4). Of these data, we have used only those which represent the DMF rates of rural or semi-rural areas among 358 United States counties.[6] We selected these data because we thought there would be less irregularity in the environmental circumstances surrounding the rural children, and that the rural children probably would be more uniform ethnologically than those of the urban centers. Furthermore, these data have served as the basis of another study for another purpose, some of the conclusions of which can be verified by the method suggested here (5).

In the paper by East and Kaiser (5) the unit of dental decay was the total DMF (decayed, missing or filled) teeth expressed per 100 children. We have suggested that this method is logically and biologically unsound, because two different statistical units are used: teeth and children. It might appear that we are making the same error in the present study, inasmuch as we relate the number of DMF teeth to groups of 100 children. We have circumvented this error by relating the number of DMF to the accumulated exposure units for the different age groups. We have converted, so to speak, the individual child into an amount of post-eruptive time and relate the number of DMF teeth in the various groups to the proper amount of exposure time.[7] For our present purpose, we have combined the DMF values for the boys and the girls since they were published separately in the Public Health Bulletin. We also determined the average exposure units as they would correspond to the age groups of the original data. These age groups were 6–8, 9–11, and 12–14. To simplify the problem, we adopted a period of 12 months as one "exposure unit" of post-eruptive tooth age. The values for the various age groups follow:

6–8	years	(average	7	years)—	11	exposure	units.
9–11	"	("	10	")—	51	"	"
12–14	"	("	13	")—	126	"	"

A unit of decay is one DMF tooth. The method of determining a DMF tooth has been described elsewhere. It will suffice to state here that a tooth must be counted as either decayed, missing (it is assumed that, considering the age of the child, the tooth has been extracted following caries) or filled (it is assumed that the tooth has been filled because of previous decay). An individual pathological (DMF) tooth cannot be counted in more than one of the above-mentioned categories.

It will be seen, as previously stated, that we now have both the numerator and denominator of our ratio in same terms, teeth. The purpose and method of our present study can be explained with one example. In Adams County, Indiana,[8] at the age of 6–8 (mean 7) the boys and girls had a combined average of 40 DMF per 100 examined children. The ratios of DMF teeth per 100

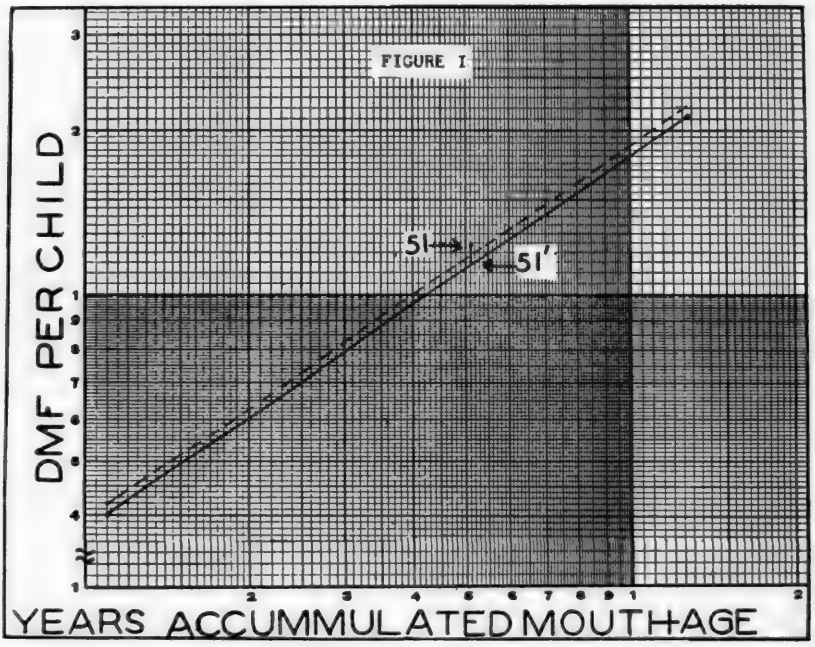
[6] In the Public Health Bulletin, localities having a population of less than 5,000 are classified as "Balance" or the rural or semi-rural areas.

[7] By this method we can even bring the original data which were determined and published as the number of events per 100 children, into a logically and biologically sound form.

[8] Page 29, *United States Public Health Bulletin* #226, 1936.

children in the 9–11 (mean 10) and the 12–14 (mean 13) age groups were 124 and 212 respectively. The frequencies were plotted as ordinates to the corresponding exposure units of 11, 51 and 126 accumulated years as abscissa on a double logarithmic paper as shown in *fig. 1*.

The 3 points 11, 51 and 126 fall nearly in a straight line. The best fit of a straight line to the 3 points could be determined by mathematical calculation and would probably closely coincide with the dotted line which we have drawn by inspection.[9] We have connected the 2 end points, 11 and 126 (accumulated post-eruptive tooth age), by a solid line. It will be noted that the solid line



would run almost parallel to the line of best fit. Each point, 11, 51 and 126, is about equidistant from the dotted line.

We are not interested in the exact position of the theoretical (dotted) line; we are concerned with its combined (accumulated) deviation from the actual values and its slope, the angle against the horizontal axis; when we assume that the theoretical (dotted) line will be almost parallel with the line (solid) connecting point 11 to point 126, the slope of both lines will be about equal. (The probable errors can be neglected for our practical purposes.) The *accumulated*

[9] We emphasize that the data with which we are working does not warrant the use of mathematical methods to locate this or any other curve, because the original material combines rather wide age groups and we have combined the 2 sexes; also the eruption tables are too meager to set definite limits as to the total post-eruptive tooth-age of our groups.

distance of the 3 points from the theoretical (dotted) line will be only slightly greater than the distance between point 51 and the solid line connecting points 11 and 126. This distance is shown in the figure as the distance between point 51 and point 51^1. If the distance between the various points and connecting lines remains the same, then we may use the distance of point 51 to point 51^1, IN THE SOLID LINE which we drew by inspection, as a criterion of goodness of fit.

The distance of point 51 from point 51^1 we measured logarithmically (relative distance) and expressed the value in per cent of the theoretical point 51^1. The measurement was made by applying point 1 of the logarithmic scale to point 51

TABLE II

Distribution of deviations of actual number of DMF permanent teeth at intermediate accumulated mouth-age (chronological age 10), when measured in per cents of theoretical values upon lines connecting values for accumulated mouth-age for children aged 7 and 13 years respectively for 358 rural United States counties

NUMBER OF COUNTIES	PER CENT DEVIATION	NUMBER OF COUNTIES	PER CENT DEVIATION
51	0	1	31–32
85	1– 2	0	33–34
45	3– 4	0	35–36
35	5– 6	1	37–38
41	7– 8	1	39–40
20	9–10	1	41–42
16	11–12	2	over 43
16	13–14		
9	15–16		
12	17–18		
7	19–20		
2	21–22		
2	23–24		
5	25–26		
5	27–28		
1	29–30		

Mean deviation = 7.3 per cent ± .43.
Standard deviation = 8.2 per cent ± .31.

of the figure and reading the distance to point 51^1 on that logarithmic scale. This gives the distance directly into percentage.

In that way we plotted the respective lines for all 358 counties and measured the distance of point 51 from point 51^1.[10] The mean deviation value amounts to 7.3 per cent. The distance of deviation of point 51 (the value of the children of ten years of age) from 51^1 was generally so small that in case a really theoretical line had been determined by the method of least squares the deviations would probably not exceed the mean error of the original points.

In Table II the deviations of the point 51 from corresponding connecting

[10] A limited supply of the entire set of 358 curves is available; apply to either author.

lines for all 358 counties of the study are given. The standard deviation is only about one-twelfth of the theoretical value, exactly 8.2 per cent. There are only a few counties in which we find a larger deviation. These deviations are not surprising when we consider that often in the original Public Health survey data there are counties where certain data are "missing." Often the number of missing teeth will be reported as "unknown." The missing data will of course affect the fit of the curve.

But in spite of the missing data the results are such that we suspect that behind this regularity of increase in caries attacked permanent teeth is to be found a general biological law. The exact nature of this law and its operation is as yet obscure, but we suggest that it merits further investigation.

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ACID PRODUCTION OF ORAL BACTERIA ASSOCIATED WITH DENTAL CARIES[1]

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A controversy still exists over the organism or organisms of the oral flora responsible for the acid that must be produced to decalcify the enamel and dentin in carious lesions. One group (1–6) holds that a single species or type (*L. acidophilus*) which not only can produce but also maintain an acid environment sufficient for *in vivo* decalcification (pH 5.0) is primarily responsible. Others (7–11) feel that the action is essentially non-specific and depends on the acidogenic powers of any one or combination of bacteria. Hence, it was thought of interest to report some simple experiments dealing with the aciduric and acidogenic properties of strains of oral lactobacilli, yeasts, streptococci, and staphylococci, which are the organisms most commonly associated with dental caries.

MATERIAL AND METHODS

For our purposes 30 strains each of yeasts and smooth lactobacilli were isolated by plating on tomato juice agar specimens of saliva from children showing clinically active caries. In a similar manner, employing blood agar, 30 strains each of streptococci and staphylococci were obtained from specimens of saliva of children with or without evidence clinically of caries activity. The streptococci were about equally divided between the alpha and gamma types. The majority of the staphylococci was without pigment. No attempt was made to use a selective medium such as described by Harrison (12) to facilitate the isolation of acidogenic streptococci. However, later trials with this medium indicated that acidogenic streptococci were not especially numerous in the samples of saliva cultured.

EXPERIMENTAL

The first experiment was designed to test the effect of acidity upon the growth of staphylococci, streptococci, and lactobacilli on tomato juice agar which is ordinarily used for estimating the number of lactobacilli in the saliva as an index of caries activity. Eighteen hour glucose broth cultures of the organisms were used and 1 loopful transferred to a tomato agar plate of a series adjusted with lactic acid or sodium hydroxide to pH 4.5, 4.7, 5.0, 5.3, and 5.5 as determined by the quinhydrone electrode. The plates were incubated at 37 C. for 96 hours and results are recorded in *fig. 1*.

Fig. 1 indicates that while most of the strains of streptococci grew readily

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at pH 5.5, only 2 were found to grow at pH 5.3. All the strains of staphylococci grew at pH 5.0 and the majority at pH 4.7. Not until pH 4.5 was reached was there a marked diminution in the growth of the staphylococci, but 25 of the 30 strains of lactobacilli were still capable of definite growth at this hydrogen-ion concentration.

The second experiment sought to obtain some information not only about the production of acid sufficient for decalcification (pH 5.0) but also what influence this acidity had upon the viability of the organisms. All 30 strains of yeast, lactobacilli, staphylococci, and streptococci were inoculated respectively into tubes of glucose broth. Viability was tested by removal of 1 loopful from each culture to fresh glucose broth at 24 hour intervals for 10 days. The appearance of turbidity in the subculture by bacteria morphologically similar to the parent culture was considered evidence of growth. Acidity of the culture

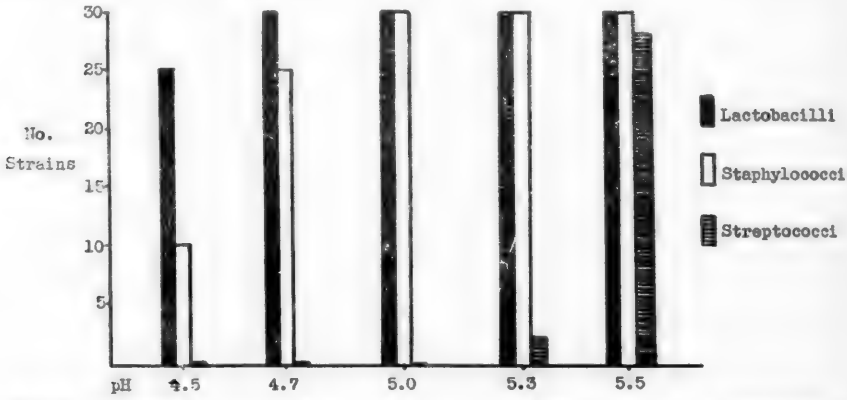

FIG. 1. Influence of acidity on growth of lactobacilli, staphylococci and streptococci on tomato juice agar

on the tenth day was determined by use of a spot plate with brom-cresol-green and brom-phenol-blue as the indicators. The data are listed in Table I.

It is obvious that the streptococci rapidly died out under the test conditions; only 2 strains survived for 10 days. In contrast all the other cultures grew readily after 10 days in the glucose broth. In respect to acid production only the lactobacilli were able to lower markedly the hydrogen-ion concentration below pH 5.0, although the streptococci formed singly or on the average slightly more acid than the staphylococci and yeasts.

A portion of this experiment was later repeated by one of us (M. L. S.) using the serum glucose agar described by Harrison (12) which was modified only by the substitution of beef for veal infusion and horse for sheep serum. Specimens of saliva were obtained from 20 children of whom half were clinically negative for caries activity. A loopful of each specimen was streaked on a plate of the medium and 2 strains of streptococci were isolated from each plate before placing in glucose broth; the acidogenic types were identified by their

yellow color or yellow zones around the colonies on the medium, and they were about equally represented with the non-acidogenic types for study. The glucose broth cultures were inoculated and incubated for 10 days at 37 C. after which a loopful was transferred to fresh glucose and serum dextrose agar. Although growth occurred in all but 3 tubes of the first series of glucose broth inoculated,

TABLE I

Acid production and viability of oral streptococci, staphylococci, yeasts and lactobacilli in 2 per cent glucose infusion broth

TYPES	NO. STRAINS	SURVIVAL IN DAYS				AVERAGE FINAL pH
		1	2	5	10	
Streptococci	30	25	8	4	2	4.6
Staphylococci	30	30	30	30	30	4.7
Yeasts	30	30	30	30	30	5.0
Lactobacilli	30	30	30	30	30	3.8

TABLE II

Acid production in Brom-cresol-green Dextrose Agar by pure cultures and mixtures of 30 strains each of oral streptococci, staphylococci, yeasts and lactobacilli

TYPES	AMOUNT INOC.	POSITIVE COLOR CHANGES IN HOURS			
		24	48	72	96
	c.c.				
Streptococci	0.2	0	0	0	0
Streptococci	0.1	0	0	3	5
Staphylococci	0.1				
Staphylococci	0.2	0	3	5	6
Streptotocci	0.1	0	0	0	0
Yeasts	0.1				
Staphylococci	0.1	0	0	2	3
Yeasts	0.1				
Yeasts	0.2	0	0	0	1
Lactobacilli	0.1	28	29	29	29
Yeasts	0.1				
Staphylococci	0.1	28	29	29	29
Lactobacilli	0.1				
Streptotocci	0.1	28	29	29	29
Lactobacilli	0.1				
Lactobacilli	0.2	29	29	29	30

no growth was observed in the second series of glucose broth or on the serum glucose agar plates.

Since Fosdick and Hansen (13) have already shown the marked increase of lactic acid by lactobacilli in the presence of yeast, a third experiment was developed to test the synergistic or symbiotic behavior of these cultures. Glucose agar, (pH 4.75),[2] containing brom-cresol-green as the indicator was used instead

[2] Supplied by the Difco Laboratories as Brom-Cresol-Green Dextrose Agar.

of glucose broth. This medium has the advantage of dispersing the bacteria of the inoculum when used as a "shake" culture and allowing easy observation. It has also been found to be as satisfactory as tomato juice agar as a laboratory method in the diagnosis of caries activity (14). Tubes containing the agar were melted, cooled to 50 C., and inoculated either with 0.2 cc. of 18 hour glucose broth cultures of the various organisms or with 0.1 cc. of one culture and 0.1 cc. of another. In this way the effects of pure cultures and the action of one species in the presence of another was tested. The tubes were rotated after inoculation to mix the contents, after which they were allowed to solidify. Incubation was carried out at 37 C. for 96 hours with daily observation. Changes in the blue-green color were considered positive only when green was no longer the dominant color and yellow took its place. The results are given in Table II. Table II is essentially self-explanatory. Neither the yeast nor the streptococci by themselves or in combination produced marked amounts of acid in 96 hours; a few strains of staphylococci did in 48 hours, but 29 of 30 strains of lactobacilli turned the medium yellow in 24 hours.

DISCUSSION

It is generally accepted that the essential mechanism of decalcification depends not only on the production by bacteria of acid in localized areas but also on the tolerance of the environment by the organisms concerned. The first part of the action has been demonstrated on many occasions and for several types of bacteria, as a result of which divergent claims for responsibility exist. References to the second requirement are not so frequent, and in the matter of actual *in vivo* decalcification little information is available, although the methods of Stephan (15) and Miller and Muntz (16) should be very helpful.

Our experiments were *in vitro* tests, and as such are open to every criticism applicable to these procedures. They do, however, emphasize once again the acidogenic and aciduric properties of the lactobacilli, which alone of the group produced on the average a hydrogen-ion concentration markedly below pH 5.0. Although the terminal acidities in glucose broth of the other organisms reached this "critical" level for decalcification or slightly below, it is difficult to assume that in the face of the neutralizing factors in the mouth these would have quantitatively sufficient ions left for the necessary calcium exchange as compared with the lactobacilli.

In a similar manner the susceptibility of the oral streptococci to acid whether of their own metabolism or in a controlled environment was illustrated. It is extremely doubtful if the rate of acid formation, which has been shown by Fosdick and Starke (17) to be considerably more rapid for yeasts and streptococci than lactobacilli is anywhere near as important in decalcification as the ability to live or reproduce in surroundings inimical to their survival. This feature may explain the failure of Miller, Muntz and Bradel (18) to obtain significant differences in the formation of lactic acid by plaque material from carious and non-carious teeth; that is, the 30 minute fermentation period of the glucose by dried plaque material only showed the fermentation by the strepto-

cocci which are numerically the dominant organism in the mouth and did not indicate the slower acting lactobacilli.

There is little or no question about the growth or survival of yeasts in an acid medium. Our strains were no exception. They produced consistently a pH of 5.0 in glucose broth, lived for 10 days in these cultures, and grew luxuriantly on the surface of the color medium. Fosdick and Wessinger (19) have reported a careful quantitative analysis of the fermentation of glucose by an oral strain of *Saccharomyces cerevisiae*, and have also shown the influence of this organism on lactic acid production by the lactobacilli. From these results they have postulated on the possible symbiotic role of the yeasts in dental caries. This theory is very attractive not only chemically but also because these forms are very frequent in carious mouths. However, there is one marked shortcoming: many, if not the majority, of these forms in the saliva are not true yeasts but are monilias (Knighton (20)). In our group of 30 strains only 2 formed ascospores on carrot plugs and could be considered belonging to Saccharomyces; the remaining strains produced soft creamy colonies on Sabouraud's medium with a tendency toward forming mycelia, features which would tend to classify them among the monilias rather than the yeasts.

Some interesting observations were made with the staphylococci of which a few strains showed definite aciduric tendencies as measured by color changes and survival in acid broth. Little attention has been paid to the staphylococci since they seem to be numerically unimportant in the salivary flora, but Snyder (21) previously pointed out that based on correlation coefficients the staphylococci approached more closely to significant figures (0.3) than either the streptococci or yeasts.

At this time an experimental approach to the problem of symbiotic or synergistic action of these forms was tried, which might test the thesis of nonspecificity held by many. The results certainly offer no conclusive evidence, but they did show that outside of the lactobacilli only a few strains of staphylococci were able to produce positive color reactions in the brom-cresol-green dextrose agar. On the other hand, no information was secured by this method whether the amount of acid produced by combinations of lactobacilli and the other organisms was greater than that formed by the lactobacilli alone.

SUMMARY

The acidogenic and aciduric properties of 30 strains each of oral lactobacilli, streptococci, staphylococci, and yeast-like organisms were tested. All strains produced a terminal pH of 5.0 or less in glucose broth, but the lactobacilli alone lowered the hydrogen-ion concentration markedly below this level. On the basis of subculture after 10 days in glucose broth all except the streptococci could be considered aciduric; only 2 of the 30 strains were capable of being subcultured at the end of this period. In brom-cresol-green dextrose agar (pH 4.75) neither the streptococci nor yeast alone or in combination changed the color in 4 days. A few strains of staphylococci gave positive reactions in 48 hours.

However, 29 of the 30 strains of lactobacilli turned the medium in 24 hours or

less. Symbiotic mixtures gave no indication of increased acid production under the conditions of the experiment.

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NUTRITIONAL REQUIREMENTS OF ORAL LACTOBACILLI

I. Pantothenic Acid and Dextrose (D-Glucose)[1]

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Diligent investigations are constantly disclosing more information about the complexities of human nutrition. From this acquired knowledge, a list of the food essentials for good health is being compiled and modifications made. The proper kind and quantity of food and drink are not only necessary for good health, but they are requisites for the maintenance of life itself. Since bacteria need many of the same nutriments as man, a study of their food essentials seems justified—not from the standpoint of nurturing bacteria thought to be harmful, but rather to reduce or nullify their growth and reproduction by the possibility either of eliminating, or making unavailable, certain required substances. This work consists of observations of growth response to increments of pantothenic acid and dextrose which were made in an attempt to evaluate possible factors governing the numerical presence of the common types of acidophilus found in the mouth.

The acidogenic bacteria are the only agents in the mouth which are capable of converting fermentable sugars to acids, thus effecting a local decalcification of tooth structure. Strains of oral lactobacilli were selected for this study because large numbers of these microorganisms are constantly present in mouths with active dental caries.

Two substances are present in the collected saliva which are required for the growth of oral lactobacilli, namely, pantothenic acid (1), which is a filtrate factor of the vitamin B complex, and dextrose. Repeated determinations of the blood pantothenic acid (2) of normal persons vary within narrow limits (about .02 gamma per cc., with a range from .19 to .32 gamma per cc.). It has been reported in a previous paper (3) that the salivary pantothenic acid values range from .012 to 0.19 gamma per cc. The amount of dextrose in the collected saliva depends largely on the nature of the diet and the degree of sugar retention in the mouth. Reducing substances in small quantities (11 to 30 mg. per 100 cc. of saliva), assumed to be glucose, have been reported as present in the salivary secretions (4), but how much of this material is actually fermentable, is not definite.

[1] Received for publication May 11, 1942.

[2] William John Gies Fellow of the American College of Dentists.

METHOD OF ISOLATION

Salivary specimens were diluted 1 to 20, and 0.1 cc. of this dilution was spread on peptone tomato juice agar (5) adjusted at pH 5.1. After 4 days of incubation at 37.5°C., typical colonies were fished out and pure cultures established. The organisms used in this experiment were Types I, II and III, classified by cell morphology and colony characteristics, as suggested by Hadley, Bunting and Delves (6). Only smooth, stable colonies were studied. Rough and variant

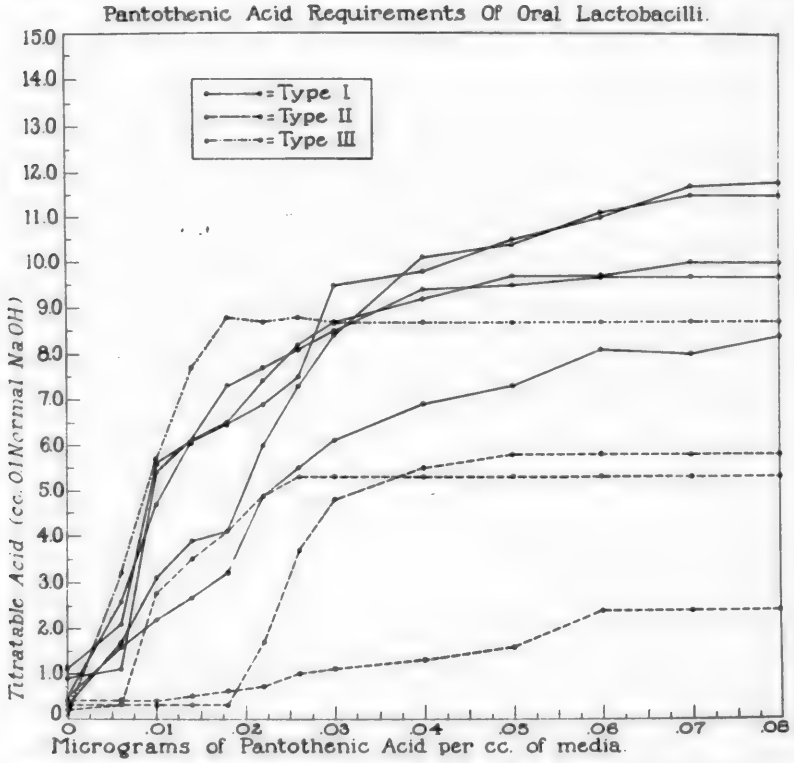

FIG. 1. Acid production plotted against increments of calcium pantothenate

colonies were not used because of their inconsistent growth in the type of medium employed.

PREPARATION OF MEDIA

Pantothenic acid-free medium as described by Pennington, Snell and Williams (7), was prepared so as to double the concentration of the final medium. The mixture was adjusted to pH 6.8 and tubed in 5 cc. quantities. Five cc. quantities of distilled water were added to the control tubes. Five cc. quantities of distilled water containing varying amounts of calcium pantothenate were added to the experimental tubes. Glucose was added to make a 1 per cent solution.

This same synthetic medium without dextrose but with an optimal quantity of calcium pantothenate (0.03 gamma per cc.) was used for the studies of dextrose requirements. Increments of dextrose were made by adding a solution of dextrose in distilled water. No dextrose was added to the control tubes. The medium was autoclaved at 15 pounds for 15 minutes and cooled to room temperature.

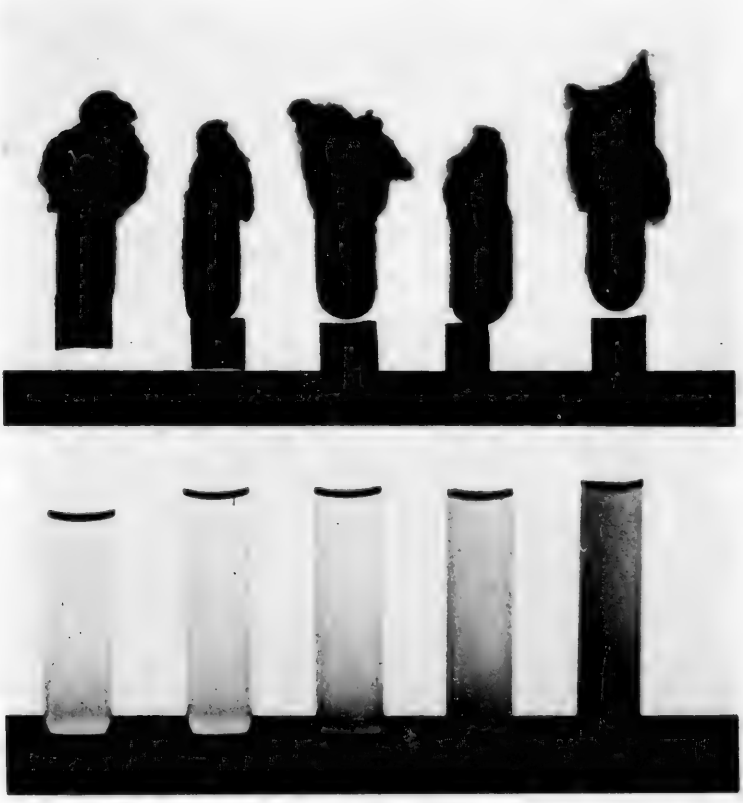

FIG. 2. Increase of turbidity resulting from bacterial growth in tubes containing increasing amounts of calcium pantothenate (increments of 0.002 micrograms per cc.) from 0.006 to 0.014 micrograms calcium pantothenate per cc. of medium. Last tube shown contains only ½ of optimal quantity of pantothenic acid.

PREPARATION OF INOCULUM

Strains of selected types of lactobacilli from 48 hour cultures of media, containing adequate amounts of pantothenic acid and dextrose, were repeatedly centrifuged and washed with 0.85 per cent saline 3 times. The final resuspension of bacteria was made in 10 cc. of saline solution. One drop of such a suspension was the inoculum.

Measurement of acid production was made by titration to pH 6.8 with 0.1

N NaOH, using .04 per cent brom-thymol blue as the indicator. Measurement of growth as indicated by a turbidimeter showed a correlation between the amount of acid formed and the multiplication of the organisms.

OBSERVATIONS

The plottings (*fig. 1.*) represent the actual titration readings obtained after incubation for 72 hours. In this synthetic medium as previously described, the dextrose content was 1 per cent, with increments of calcium pantothenate as indicated on the abscissa. Each line represents an individual strain.

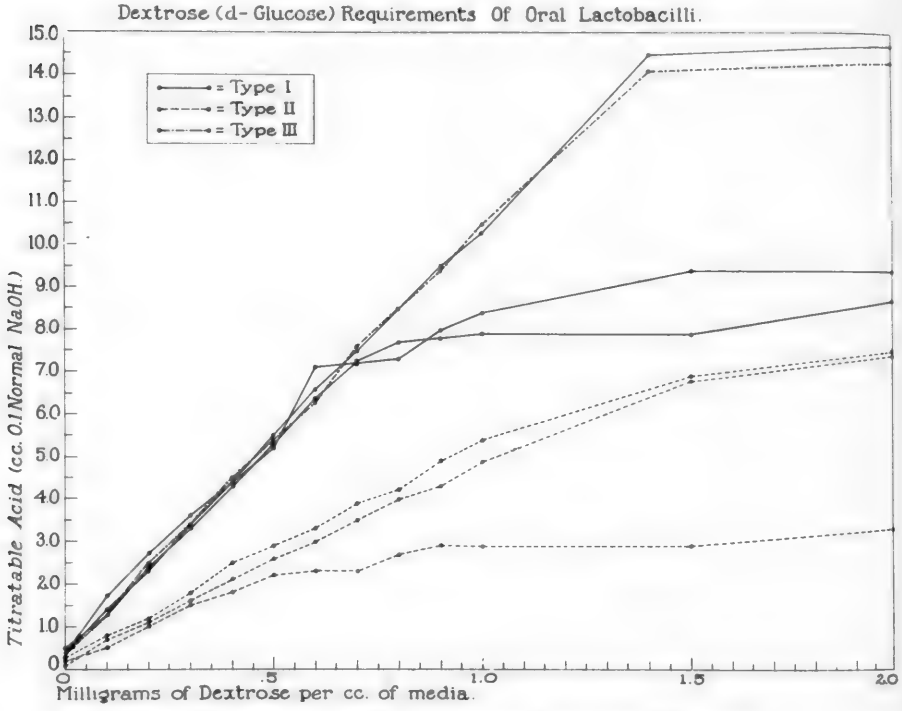

FIG. 3. Acid production plotted against increments of dextrose

Snell, Strong and Peterson (8) have shown that pantothenic acid is an essential growth factor for lactic and for propionic acid-forming bacteria. It will be noted that small amounts of acid were produced in the control tubes. There are 2 ways in which such traces of acid could be formed—1, the fermentation by the enzymes of the bacteria in the inoculum without bacterial reproduction, and 2, the presence of traces of pantothenic acid in the inoculum. Such traces present after repeated washings were probably due to the inability to remove all of the pantothenic acid from the organisms which had been grown in a medium containing adequate amounts of pantothenic acid.

It has been reported (6) that there is a variation of acid production among the groups of oral lactobacilli. Under identical cultural conditions, this graph indi-

cates that Types I and III produce more acid than Type II. It also shows the variation in total acid production of the different strains among the types. Repeated experimentation with each strain gave identical total acid production.

The variation in the rise of these curves shows that with equal concentrations of pantothenic acid, the acid produced by these types shows great differences, which are not due to the rate of acid production by these bacteria but are due to the rate of reproduction of these bacteria at this concentration. This was confirmed by measuring the turbidity of these cultures and the correlation of such turbidities with acid production (*fig. 2.*).

The graph on dextrose requirements of oral lactobacilli shows increasing amounts of dextrose on the abscissa and the resultant amounts of acid production on the ordinate (*fig. 3.*). These results are also actual readings from titrations with 0.1 N NaOH. With a given amount of dextrose, Types I and III show more growth and consequently produce more acid than Type II. The increased growth of these strains is proportionate to the amount of dextrose added up to 1.5 mg. per cc. of medium. Additional amounts of dextrose far beyond the amount included on this graph did not materially increase the rate of growth as indicated by acid production. This emphasizes the meager sugar requirement of these organisms in vitro.

CONCLUSIONS

1. Pantothenic acid is a requirement for growth of oral lactobacilli.
2. Pantothenic acid is present in human saliva.
3. The optimal quantity of pantothenic acid for oral lactobacilli growth is not greater than usually found in human saliva.
4. Dextrose is a requirement for growth of oral lactobacilli.
5. 1.5 mg. of dextrose per cc. of medium is an adequate amount for maximum growth of oral lactobacilli.
6. The pantothenic acid and dextrose requirements for maximum growth of oral lactobacilli vary for different types and strains.

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FORM OF CONTACT SURFACES AND THEIR RELATIONSHIP TO CARIES SUSCEPTIBILITY[1]

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It is apparent from the observations and statements of the majority of investigators that the typical tooth, upon eruption, is rounded or convex in form at the surface that makes proximal contact with adjoining or proximal teeth (1). It is assumed, therefore, that in newly erupted teeth the proximal surfaces have a marble like contact that is designated as the contact point.

After eruption, wear starts immediately at the contact surface due to friction caused by the continued movement of the teeth in their sockets. Worn or flat planes are found. A microscopic study of the contact surfaces will demonstrate the concave surfaces and other varied fo ...s caused by friction from the adjoining tooth and thus will show that the contact surface of one tooth may be more resistant to wear than the contact surface of an adjoining tooth (*figs. 1–4*, p. 474).

It is generally believed that caries usually starts below the contact point when no plane exists (2), but as soon as a plane or concave surface is formed frontal or undermining caries may be found on the altered surface. Beust concluded that interproximal caries (3) of enamel begins, in practically all cases, at the actual point of contact of a tooth with its neighbor. He also stated that the presence of an acid-secreting plaque or colony at this point is environmentally and mechanically impossible.

It is possible that caries may start below the contact point previous to the wearing down of the contact surface by friction. However, caries is found within the contact surfaces. Miller (4) noted that teeth with convex surfaces which touch at one point are more free from attack than those with flat or slightly convex surfaces.

The statement that the friction caused by the rubbing of the contact surfaces prevents caries is not true. It is apparent that rubbing or friction of the contact surfaces cannot prevent caries.

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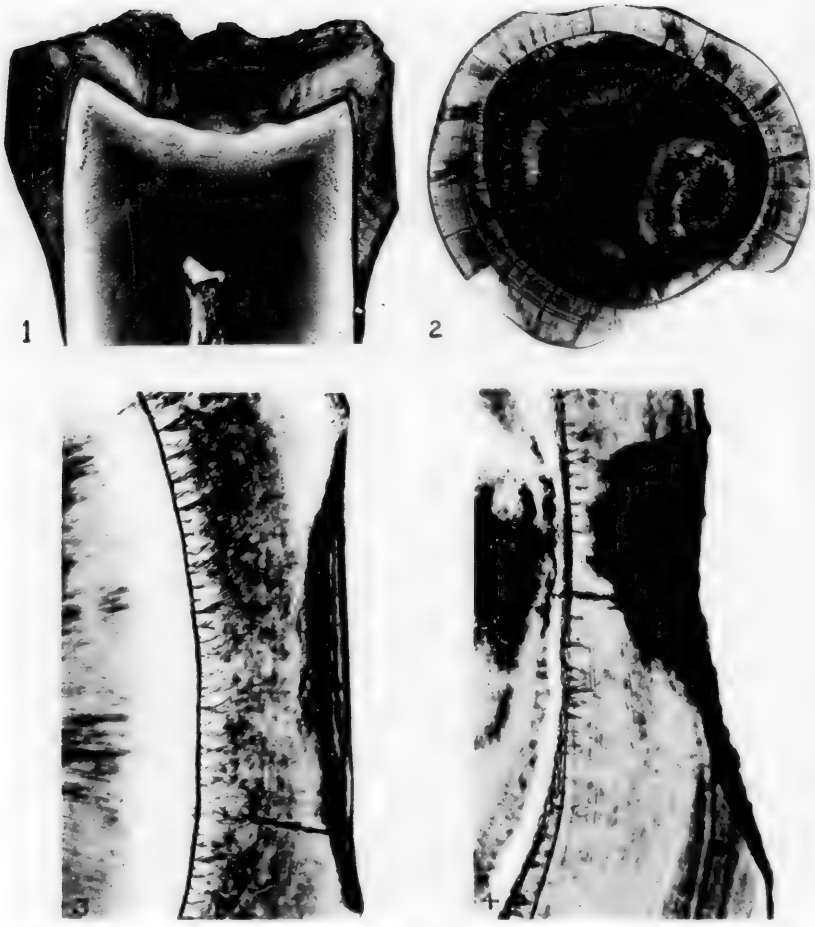

FIG. 1. Plane contact surface on left and concave contact surface on right side
FIG. 2. Irregular contact surfaces on both sides
FIG. 3. Concave surface with enamel caries limited within surface
FIG. 4. Concavity of contact surface and dental caries within that surface

RESISTANCE OF ENAMEL TO DENTAL CARIES[1]

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REVIEW OF LITERATURE

Pickerill (1) reported on the comparison of permeability of teeth of the Maori and English people. He found that before eruption the permeability to silver nitrate of the tooth structure of Maori and English was the same. However, after eruption the Maori teeth became impermeable much sooner than English teeth, a finding which Pickerill regarded significant in the reduced incidence of caries among uncivilized people.

Gottlieb (2) showed that there is more organic substance present in the pits and fissures and on the proximal surfaces than in other areas of human and monkey teeth. He associated this finding with the distribution of carious areas. Gottlieb (3) showed that the entrance of lamellae may become hornified, preventing invasion by microorganisms. He also demonstrated the progress of undermining caries through the lamellae.

Mellanby (4) demonstrated that a more homogeneous and better calcification of enamel with a resulting increased resistance to dental caries may be obtained by the administration of vitamin D during the development of the teeth.

Barker (5) illustrated a case of caries progressing along a lamella to a point in the enamel where the lamella came to an abrupt halt on reaching an area of increased calcification, or transparent enamel. Here caries stopped demonstrating that the spread of caries may be stopped by transparent enamel.

Kanner (6) succeeded in staining the enamel of teeth in the mouth by application of polychrome methylene blue on the enamel surface for 5 to 30 minutes. The enamel was diffusely stained and the stain ended abruptly at the dentinoenamel junction.

Barnum and Armstrong (8) investigated the entrance of radioactive phosphorus into enamel of extracted teeth. The amount entering differed in the 3 groups studied and, they suggested that variation of the size and capacity of enamel channels may be responsible.

FINDINGS

Extracted teeth, which had been kept in 5 per cent formalin were dipped into ammoniacal silver nitrate for 3 to 5 minutes and then into 5 per cent formalin

[1] Presented at the 20th General Meeting of the International Association for Dental Research New York, March 14-15, 1942 (J. D. Res. **21**: 317, 1942). Received for publication May 25, 1942.

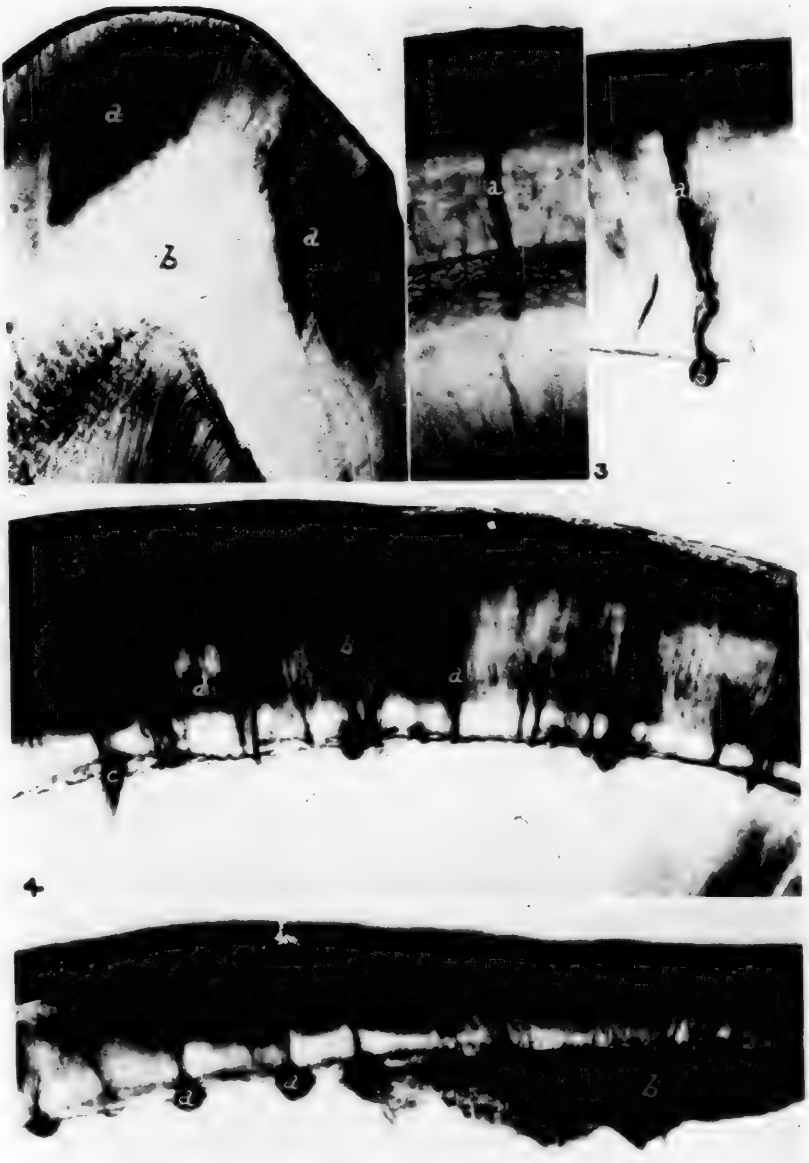

FIG. 1. Tooth impregnated with ammoniacal silver nitrate. Prism sheaths stained with silver nitrate (a); transparent enamel, not stained (b).

FIG. 2. Tooth impregnated with ammoniacal silver nitrate. Peripheral part of enamel stained. Lamella (a), stained all throughout.

FIG. 3. Tooth impregnated with ammoniacal silver nitrate. Peripheral part of enamel stained thoroughly, prism sheaths stained in middle part, in the dentinal part only some tufts stained. Lamella (a), carried the stain to dentin (b).

FIG. 4. Tooth impregnated with ammoniacal silver nitrate. Peripheral part of enamel thoroughly stained. In middle part, (d), prism sheaths are stained. Dentinal part of enamel is unstained. Tufts, (a), stained. Lamella (b), carried the stain to the dentin (c).

FIG. 5. Tooth impregnated with ammoniacal silver nitrate. Isolated spots in dentin (a). Large area formed by penetration of silver nitrate through enamel into dentin (b).

for 3 minutes. Other teeth were impregnated before extraction. Ground sections were prepared.

In accordance with the findings of Barnum and Armstrong, there was considerable variation in reaction of different teeth. The first significant difference was that teeth impregnated before extraction were less permeable than teeth impregnated after extraction. Furthermore, a great difference in permeability of extracted teeth was noticed. Silver nitrate penetrated organic parts only and did not penetrate through calcified areas. Areas of transparent enamel remain light while the uncalcified prism sheaths, susceptible to silver nitrate penetration, are stained. *Fig. 1*, shows such an area of unstained transparent enamel amid the surrounding sheaths which have taken up the silver nitrate.

The lamellae also show differences in susceptibility to silver nitrate penetration. In all cases compared, the teeth were treated the same way. In some teeth the lamellae stained with difficulty while in others they stained readily. *Fig. 2* shows a lamella stained very darkly, evidence of thorough penetration of silver nitrate. *Fig. 3* shows a black spot of silver nitrate in the dentin where it is contacted by a lamella. *Fig. 4* shows several black spots where silver nitrate entered the dentin apparently by the lamella pathways of the prism sheaths in the tufts. *Fig. 5* shows a large area of silver nitrate stain apparently formed through the confluence of numerous smaller areas.

DISCUSSION

If a tissue is resistant to the action of acid it does not necessarily follow that it should be resistant to proteolytic action. Acid action on enamel is not considered as caries of enamel by the author. Acid action first produces a chalky appearance on the surface of the enamel with dissolution following. Neither of these stages are called caries by the author. The process is only considered to be caries when there is an invasion of the organic matter of the tooth structure (in our particular case, enamel) by proteolytic microorganisms. Enamel contains 3 different forms of organic matter, prism sheaths, lamellae, and tufts. Since caries starts on the surface of the tooth and the tufts do not reach the surface, the tufts are important only in the spreading of caries, and may be considered of no importance here. However, either the prism sheaths or the lamellae may be invaded from the surface. It is possible for the prism sheaths to become calcified producing an area of more homogeneous calcification, transparent enamel. This calcified area is much more resistant to the invasion of microorganisms than are the uncalcified sheaths. As far as the lamellae are concerned especially those derived from cracks during the development of the tooth, there is no possibility for calcification. One way by which these passages may be obstructed is by the hornification of their entrances but it appears that there may be other means of obstructing the lamellae. Experiments on penetration of silver nitrate point to the possibility of making the lamellae less permeable and consequently more resistant to invasion by microorganisms. Possibly some element or elements in the saliva may impregnate enamel lamellae and increase their resistance.

The more rapid penetration of teeth treated after extraction than those treated before extraction is probably due to the fact silver nitrate penetrates dead organic matter more easily. Silver nitrate and proteolytic bacteria seem to favor the same type of tissue. The observation that silver nitrate cannot penetrate completely calcified enamel (transparent enamel) coincides with Barker's finding that caries did not spread over an area of transparent enamel.

A differentiation should be made between *frontal* and *undermining caries*. In the first, caries spreads along the organic avenues of the prism sheaths. The second develops where caries reaches the dentin-enamel junction, in many cases by way of enamel lamellae.

A great variation in the permeability of lamellae to silver nitrate has been observed. Some are not affected by silver nitrate at all while others take it up in great quantities.

Remembering that the behavior of silver nitrate and microorganisms are similar, the results of this experiment indicate that enamel lamellae vary in their resistance to the passage of microorganisms and this difference in possibilities for invasion may explain varying caries incidence in different places. This indicates a possible approach to effective prophylaxis for dental caries. The various pathways mentioned must be rendered impassable for microorganisms.

SUMMARY

Caries of enamel is defined as the invasion of the organic matter of the enamel by proteolytic organisms.

There are some significant differences in the permeability of different teeth to silver nitrate. This becomes of importance because silver nitrate and the caries may progress along the same pathways, the prism sheaths and enamel lamellae.

Some areas of enamel are better calcified than others. In some cases the prism sheaths become calcified. This variation may occur between different places in the same tooth as well as between different teeth. These areas of better calcification are highly resistant to dental caries.

Some enamel lamellae are protected against invasion of microorganisms by hornification of the entrances. It is possible that the lamellae may also be protected by their impregnation with some protecting element of the saliva.

Dental caries may be prevented if the prism sheaths and enamel lamellae are rendered impassable to microorganisms.

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TISSUE REACTIONS IN EXPERIMENTAL TOOTH FRACTURE[1]

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INTRODUCTION

This report deals with fractures which were produced in the teeth of young dogs, in order that material might be made available for a study of the tissue reactions which occur in the tooth and surrounding tissues under such conditions.

Hopewell-Smith (1) referred to the reactions which occur in fractures of human teeth and noted that either fibrous union of the fragments may occur or fragments may be united by a calcific deposit. He further described the proliferation of epithelium to cover the exposed pulp and in addition postulates the probable reactions of the pulp of fractured teeth as consisting of 1, hemorrhage, 2, inflammatory symptoms, 3, necrosis, and 4, fibrous and calcific replacement. Gottlieb (2) in examining a tooth fracture of several years' standing, observes fibrous union of the fragments, the presence of bone spicules in the pulp cavity and cementum lining the pulp cavity in the region of the fracture. Boulger (3) reported essentially the same reaction in a root fracture with the added observation that a new foramen was established by the growth of cementum. He also noted that the fragments were covered by a thick investment of cementum. Boulger (4) also described a coronal fracture in which he observed a proliferation of epithelium which covered the pulp. Pritchard (5) described in a fracture of two years' duration, the appearance of fragments covered with cementum, a fibrous union, and some repair by means of secondary dentin. Kronfeld (6), in the examination of a fractured incisor, observed essentially the same reactions as those described by Pritchard. Coolidge (7) noted certain changes of a reparative nature which occur in the apical cementum of a tooth which was detached as a result of trauma. Claflin (8) in a study of the fate of root fragments in the dog observed that the pulp when present remains vital and that the tissues close over the fragments without the production of inflammatory reactions.

An examination of the literature in regard to tooth fracture reveals that most studies have been made on extracted specimens. Previous investigators report that exposure of the pulp coronally may lead to a "capping" reaction. Fractures in the region of the crown are frequently infected and the tissues in contact with such a fracture are subject to inflammation and necrosis. Localized areas of the pulp undergo calcific and fibrous degeneration. Union of root fragments has been described as fibrous or calcified. The calcified union may be cementum,

[1] Presented at the 20th General Meeting of the International Association for Dental Research, New York, March 14–15, 1942. (*J. D. Res.*, **21**: 327, 1942.) Received for publication May 7, 1942.

a modified dentin, or a non-specific calcified deposit. Very few studies have been made on the effect of fracture on the tooth and all of the surrounding tissues.

MATERIAL AND METHODS

Several mandibular deciduous molar teeth of dogs 6 to 8 weeks old were fractured by means of a forceps. The animals were then maintained for 3 weeks without any treatment and sacrificed. At this time, a segment of the jaw containing the fractured tooth and surrounding structures was removed and fixed in Zenker-formol. Following this, the tissues were decalcified, embedded in celloidin, cut at 10 mu and stained with hematoxylin and eosin.

Of the several fractures and reactions observed we have chosen for purposes of description one specimen which illustrates all of the typical conditions prevalent in our material.

The fractures produced in this specimen were as follows: 1, a coronal fracture exposing the pulp, 2, an oblique fracture extending from the gingiva to the lower half of the root, communicating with the pulp, 3, a longitudinal and transverse fracture of the apex of the root, 4, several transverse fractures which were distributed in the cervical and apical regions of the tooth. These latter fractures illustrate the various positions which fragments may assume following fracture of the tooth.

OBSERVATIONS

General Relationships

In preparing sections of the fractured tooth retained in its normal position, it was possible to observe the effect of the tissue changes in the tooth itself and also in all of the surrounding tissues, such as the gingiva, periodontal membrane, alveolus and the permanent tooth germs.

Figs. 10–12 represent selected sections of the fractured tooth and surrounding tissues which illustrate some of the important relationships which were observed in this study. Examination of these figures shows the location and type of some of the typical fractures which were produced, the relation of the displaced fragments to the surrounding tissues and the proximity of the fractured root to the permanent tooth germ.

Tissue Reactions

Epithelium: In the coronal part of the tooth the gingival epithelium has proliferated over the exposed pulp surface forming thereby a barrier for the protection of the underlying tissues. This phenomenon is sometimes referred to as pulp capping. In this tooth, the epithelium has also migrated across the pulp in one root to the connective tissue underlying the root in the coronal region as well. The extent to which the epithelial growth has occurred as well as the derivation of this tissue is clearly shown in *figs.* 1 and 2.

Pulp: The coronal part of the pulp shows evidence of previous hemorrhage which is now organized. The coronal half of the pulp chamber has been invaded

by round cells and a considerable fibrous degeneration has taken place (*fig.* 1). In some areas the odontoblasts have degenerated, apparently as a result of inflammation. Certain other changes which appear in the pulp consist of a slight hyperemia, a vacuolation of certain areas (*fig.* 3), and the occurrence of nonspecific calcific deposits (*fig.* 9). The apical part of the pulp seems by comparison with the pulp of an adjacent tooth to be relatively normal.

Reactions of Fractures

A. *Longitudinal Fractures:* 1. A longitudinal fracture extends through the crown to the pulp, fragments not widely displaced. The area between the fragments of the root in this fracture is now filled with degenerating round cells. It also contains some fibrous elements and, as shown in *fig.* 3, the tissue appears necrotic.

2. An extensive longitudinal and transverse fracture which does not continue into the pulp is located in the apical part of the root. The area between the root fragments in this specimen is completely filled with a dense fibrous stroma. In addition, there appears to have been some resorption of the fractured surfaces preparatory to a slight amount of cementum deposition. This fracture is shown in *fig.* 4.

B. *Transverse Fractures:* The transverse fractures are divided into the following groups: a, fragments in alignment but separated rather widely, b, fragments in alignment and in close apposition, c, fragments which are not in alignment.

1. An example of type "a", a transverse coronal fracture, the fragments of which are in alignment but widely separated is shown in *fig.* 1. The fragments are enclosed by epithelium but the intervening space between the two fragments is filled with dense fibrous connective tissue which is derived from the periodontal membrane.

2. A radicular fracture of a similar type is illustrated by *fig.* 7. Here again, the fibers of the periodontal membrane have filled in the space between the ends of the fragments. Some resorption of the dentin is apparent and in the upper fragment, a considerable amount of osteoid type dentin has been deposited on the pulpal surface.

3. The tissue reactions which occurred in transverse fractures which were in alignment and in which the fragments were in close proximity to one another are as follows: The fracture illustrated by *fig.* 5, which is typical of this condition, shows that a slight degree of resorption of the free surfaces occurred. Further, the periodontal fibers have almost completely filled in the space between the fragments, affording thereby a good fibrous union. In addition, a considerable amount of osteoid-like tissue has developed on the pulpal surface, at the line of fracture. This tissue as shown in *fig.* 5 closely resembles a spicule of bone surrounded by cells resembling osteoblasts.

4. Another reaction is shown in *fig.* 6. In this transverse radicular fracture, 3 marked changes have occurred: a, The ends of the fragments are covered by a thin layer of cementum and the intervening space is now filled by a large blood vessel which has established a communication between the pulp and the perio-

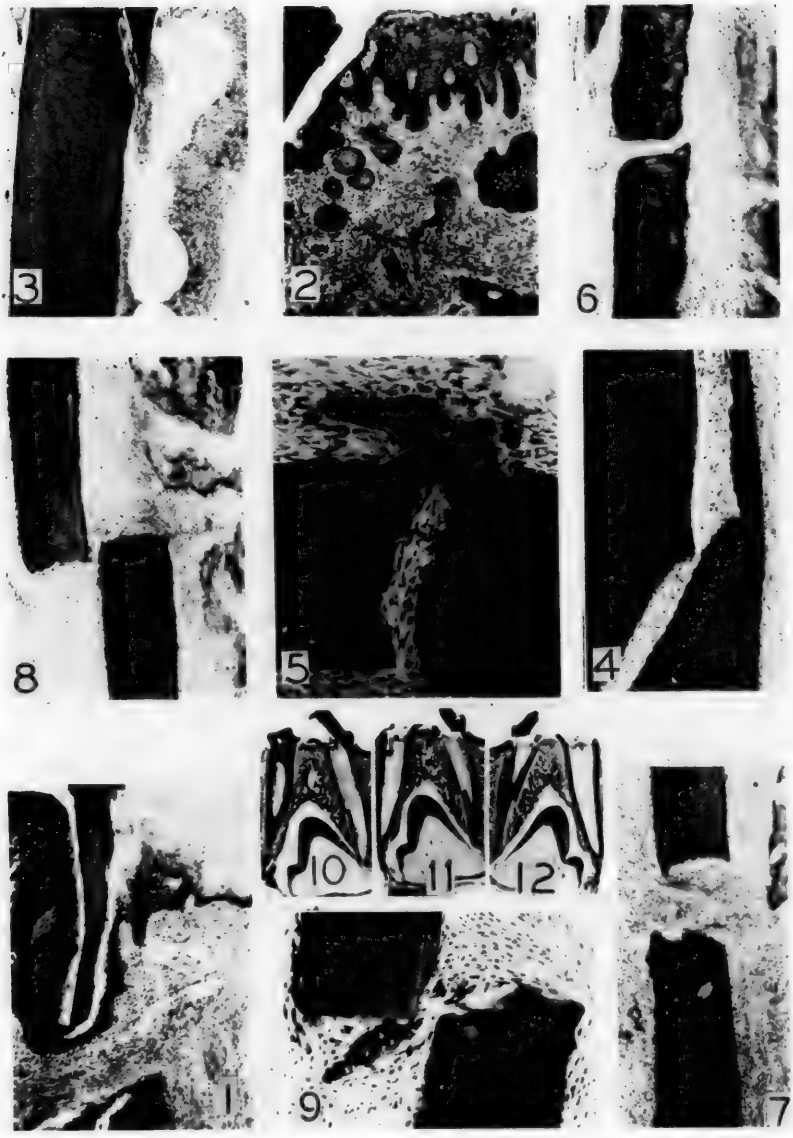

FIG. 1. Relation of gingiva to a transverse coronal fracture in which gingival epithelium has proliferated to cover exposed pulp.

FIG. 2. More extensive proliferation of gingival epithelium in situation similar to that shown in *fig.* 1.

FIG. 3. Reaction of tissues in a longitudinal fracture which extends from gingiva to pulp.

FIG. 4. Longitudinal-transverse fracture of apical part of root showing fibrous union.

FIG. 5. Transverse root fracture in which a modified dentin repair has taken place.

FIG. 6. Transverse radicular fracture in which new vascular channel has been established, extensive cementum replacement on alveolar surface and secondary dentin on pulpal side.

dontal membrane; b, extensive cellular cementum growth has taken place on the periphery of the root; and c, secondary (osteoid-like) dentin appears on the pulpal surface.

5. *Overlap Fractures:* Two radicular fractures in which the fragments overlap are shown in *figs.* 8 and 9. In both of these specimens resorption of the free ends of the fragments has taken place and a fibrous attachment of the fragment which is in contact with the periodontal membrane has also been established. *Fig.* 9 also shows the formation of some calcific deposits at the site of the fracture. *Fig.* 8 illustrates the shift which has occurred in the alveolus adjacent to a laterally displaced fragment of the root. It is apparent from an examination of this figure that resorption of the alveolus has taken place in an attempt to maintain the same relative width of the periodontal membrane throughout the axis of the root.

6. *Small Detached Fragments:* In severe fractures such as those previously described, splinters of dentin are often displaced and come to lie either in the pulp or external to the tooth. Many of these spicules were present in the pulp and adjacent tissues of the sections which were examined. So far as could be ascertained, they undergo no change.

In one of the roots shown in *fig.* 12, a fracture of the apex occurred which simulates the situation which exists in the case of a retained root fragment. In this specimen, the surface of the coronal fragment is covered by a thick layer of cementum, dense fibrous connective tissue intervenes between the fragments, and the isolated fragment of root has not caused any reactions of a pathological nature.

7. *Gingiva:* The gingiva in the area of the coronal fracture shows 2 marked changes. The first, already referred to, consists of a proliferation between the coronal fragments. The second, as shown in sections, consists in the presence of a moderate degree of inflammation comparable to that observed in a chronic gingivitis. In addition, some destruction of the epithelial attachment has taken place (*fig.* 1).

8. *Periodontal Membrane and Alveolus:* In the gingival region, the periodontal membrane shows a mild inflammatory reaction. In other areas adjacent to fractures, there is evidence of previous hemorrhage now, however, completely organized. In one apical fracture, a new vascular channel was established; in the region of the various other fractures, the membrane formed a fibrous union with one or both fragments. In the situations where lateral displacement of fragments occurred, there appears a compensatory resorption of the alveolus to

FIG. 7. Transverse fracture in which fragments are widely separated. 1—fibrous union, 2—resorption of ends of fragments, and 3—deposition of osteoid-like dentin.

FIG. 8. Overlap fracture showing 1—fibrous attachment of the lateral fragment, and 2—compensatory resorption of the alveolus.

FIG. 9. Overlap fracture in which formation of a non-organized calcific deposit occurs in region of fracture.

FIGS. 10, 11, 12. Low magnification photographs which indicate general topographical relationships of fractured tooth and surrounding tissues.

allow for the maintenance of a normal periodontal space. On the whole, the apical part of the membrane appears to be more resistant, or less subject to inflammatory changes, than the gingival region.

9. *The Permanent Tooth Germ:* As shown in *fig.* 10, one of the deciduous roots was slightly displaced, causing it to come into contact with the enamel epithelium of the adjacent permanent tooth germ. As a result of this contact, there has been a destruction of the enamel epithelium with some arrest in development of the enamel in this area. Despite the proximity of the roots to the tooth germs, there is in general relatively little traumatization of these developing teeth.

DISCUSSION

In the study of the several fractures which occurred in this material, it is noted that in regard to the reaction of the pulp the indications are that following the hemorrhage which occurs at the time of fracture the subsequent reactions depend upon local factors. In coronal areas subjected to exposure, the epithelium derived from the gingiva proliferates over the exposed surface. The coronal part of the pulp itself contains a large number of round cells and fibrous degeneration also occurs.

The reactions observed at the site of the fractures also differ to some degree depending on local factors. In general, coronal fractures are more likely to show inflammatory changes and necrosis and accordingly do not heal as rapidly as the fractures in the apical part of the tooth.

Fractures located well below the epithelial attachment are restored to functional adaptation to the greatest extent when the fragments are in apposition and in alignment, to a lesser extent when the fragments overlap, and least of all when the fragments are separated widely. In general, the first reparative reaction in the region of the fragments seems to be a resorption of the free ends of the dentin. This is followed by a deposition of a thin line of cementum and the establishment of a fibrous union.

The formation of a callus similar to that observed in the healing of a long bone fracture does not occur. Nevertheless, an attempt to produce a functional callus-like structure does appear in those fractures in which the conditions are favorable; viz., good alignment, close proximity of the fragments and vital tissue elements in this area. In contrast to the bone callus, which forms peripherally around a fracture, the deposition of osteoid tissue occurs only on the pulpal surface of the root fracture.

One reaction observed, which appears to be characteristic in the fractures regardless of the position of the fragments is the production of a calcified zone on the pulpal side of the root. This zone has been described previously as bone and cementum. In these preparations this tissue appears to be cellular dentin. It seems of interest to note that this tissue is first deposited some distance away from the line of fracture. In this respect, a situation analagous to that which occurs in the healing of a bone fracture exists.

This study shows that the striking difference between the reactions which take place typically in fractured bones and the fractured roots is that in the former a

great amount of new bone is laid down rapidly; in the tooth, a modified calcified tissue is deposited only under favorable conditions, and then to an extremely limited extent.

SUMMARY

1. The tissue reactions of deciduous molars, which were experimentally fractured, and surrounding tissues of dogs were observed.

2. The reaction of the coronal part of the pulp exposed to the oral fluids by fracture is severe; necrosis and resorption of the tissue occurrs.

3. The coronal part of the pulp which is protected by an epithelial overgrowth derived from the gingiva shows moderate round cell infiltration and fibrous replacement. The apical part of the pulp remains relatively normal.

4. The reaction which occurs in a fracture appears to be dependent on 2 factors: 1, The proximity to the oral cavity and 2, the relation of the fragment surfaces to each other.

5. Coronal fractures containing round cells and necrotic tissue do not repair as rapidly as apical fractures not complicated by inflammatory changes.

6. Fragments in apposition and in proper alignment more nearly approximate a characteristic bone repair than those in which the fragments do not meet these conditions. In these latter situations, only a fibrous union occurs.

7. In some respects, the reactions observed in a fracture of the root are similar to those observed in bone; calcific replacement, however, is not as rapid nor as extensive as is the case in the repair of a fracture of bone.

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INFLUENCE OF LOSS OF PERMANENT FIRST MOLAR ON POSITION OF ERUPTION OF SECOND PREMOLAR[1]

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An examination was made of boys and girls age 15 to 19 years inclusive, pupils of the vocational high schools in the City of New York, who had lost one or more of their permanent first molar teeth and in whom the space left by the extraction was subsequently eliminated by the shifting of the adjacent teeth. Those patients in whom the space was not entirely eliminated were not included in this survey. Measurements of distal shifting of premolars and mesial shifting of second molars was made in millimeters and correlated to the age of the patient at the time when the extraction occurred.

In previous correlations[2] it was shown that the rate of closure of the space after first molar extraction is faster in the maxilla than in the mandible, and that the tendency for the space to become entirely closed was also greater in the maxilla than in the mandible. The manner in which distal shifting of the first and second premolar teeth takes place in the maxilla is different from that in the mandible. The tendency for the mandibular premolars is to shift singly, the second premolar begins to shift sooner and travels faster than the first premolar. In the maxilla the premolars shift distally in unison. Although there is a disturbance at the point of contact between the first and second maxillary premolar teeth, there was no marked increase in space found between the first and second premolars in cases where the space of the extracted permanent first molar had completely closed (Table I).

The amount of space between the first and second premolars in the mandible, in cases where the space left by the extracted permanent first molar had entirely closed, showed a tendency to increase as the interval of time since the extraction of the first molar increased. In the maxilla, any existing space between the premolars showed a tendency to decrease as the interval since the first molar extraction increased (Table II).

The amount of distal shifting of the second premolars in the maxilla in those cases where the space had been entirely closed was found in 77.7 per cent of cases to be 3 mms. or less, none showing more than 5 mms. (Table III). In the mandible, only 17.5 per cent showed distal shifting of the second premolar teeth

[1] Presented at the New York Section of the International Association for Dental Research, June 4, 1941 and the 20th General Meeting of the International Association for Dental Research, New York, March 14–15, 1942. (*J. D. Res.* **21**: 300, 1942.) Received for publication May 11, 1942.

[2] Salzmann, J. A., Variation in tooth position following extraction of first permanent molars in relation to incidence and distribution of dental caries. (*J. D. Res.* **19**: 17, 1940.)

to be only 3 mms. or less, the distance being greater than 3 mms. in 82.5 per cent of these cases. A total of 33.3 per cent of the cases examined in the mandible showed from 10 to 15 mms. of distal shifting of the second premolars (Table IV).

When the amount of distal shifting was related to the age of the patient at the time when the mandibular first molar extraction occurred, it was found that when the permanent first molar extraction took place during ages 7 to 8 years,

TABLE I

Amount of Space Found Between Maxillary Pm 1 and Pm 2 After Closure of Space Left When M 1 Was Extracted Related to Age of Patient at the Time When the M 1 Extraction Occurred

AGE AT M 1 EXTRACTION	SPACE IN MILLIMETERS										
	0	1	2	3	4	5	6	7	8	9	10
years											
7	5										
8	7										
9	7										
10	14										
11	14										
12	11										
13	8										
14											

TABLE II

Amount of Space Found Between Mandibular Pm 1 and Pm 2 After Closure of Space Left When M 1 Was Extracted Related to Age of Patient at the Time When the M 1 Extraction Occurred

AGE AT M 1 EXTRACTION	SPACE IN MILLIMETERS										
	0	1	2	3	4	5	6	7	8	9	10
years											
7	1					1		1	3		1
8	4				1		2	4	2	1	
9	6		1					1	1	1	
10	10	2	3	1	1	1		8	4	2	
11	21	3	1					2	4	2	
12	14	3	4			1		1			
13	9	1	1			1					
14	5										

before premolar eruption occurs, over 90 per cent showed from 10 to 14 mms. or more of distal shifting of the second premolar teeth when examined between age 15 to 19 years. If the first permanent molar was extracted during the ages 9 and 10 years, when progress of the premolar eruption is considerably advanced, 45.2 per cent showed distal shifting of the second premolar teeth of 10 to 14 mms. or more at age 15 to 19 years. Those who had permanent first molar teeth extracted between 11 and 12 years of age, when the premolars had erupted or were just

about to erupt, showed only 11.4 per cent of second molars with more than 10 mms. of distal shifting at age 15 to 19 years. Patients who had lost first molar teeth at 13 and 14 years of age showed no second premolars with distal shifting of more than 7 mms., 82 per cent showing 5 mms. or less of distal shifting of the premolar teeth in the last age group. Thus, the amount of space between the first and second mandibular premolars varied inversely with the age of the patient at the time of extraction.

TABLE III

Amount of Distal Movement of Maxillary Pm 2 in Mm.'s Related to the Age of the Patient When the M 1 Extraction Occurred

AGE AT M 1 EXTRACTION	DISTANCE OF MILLIMETERS							
	1	2	3	4	5	6	7	8
years								
7	2	1	1	1				
8	3	1	2	1				
9	1	4	1		1			
10	4	1	6	2	1			
11	1	7	2	2	2			
12	2	4	2	3				
13	1	3	2	1	1			
14								

TABLE IV

Amount of Distal Movement of Mandibular Pm 2 in Mm.'s Related to the Age of the Patient When the M 1 Extraction Occurred

AGE AT M 1 EXTRACTION	DISTANCE IN MILLIMETERS													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
years														
7											1		1	5
8		1				1				3	1	2	3	3
9		1	3	2				1					1	2
10		1	1	1	5	7			1		3		8	5
11		4	5	6	4	6							2	2
12		1	4	3	8	2	3				1		1	
13			2	4	4	1	1							
14				2	2	1								

In the maxilla, no appreciable space was found to exist between the first and second premolar teeth in those patients who had lost first molars, but in whom the first molar space had been completely eliminated. In the mandible, a space of 6 mms. or more was found in 66.6 per cent of children with first molar teeth extracted at age 7 and 8 years. A space of 6 mms. or more was shown by 40 per cent whose first molars were extracted at age 9 and 10 years. Only 9.6 per cent of those whose first molars were extracted at age 11 and 12 years showed a space

of 6 mms. or more between their first and second premolars. Those who lost their first molar teeth at age 13 and 14 years showed no spacing between the premolars greater than 5 mms.

DISCUSSION

It should be remembered that all of the extracted first molar space had been eliminated in all of the cases examined. However, the amount of distal shifting of the premolars, the amount of mesial shifting of the second molars, as well as the amount of space between the premolars themselves, showed a direct relationship to the age of the patient at the time when the extraction occurred. Cases in which the mandibular permanent first molar was extracted at ages 7 and 8 years showed few with the second premolars less than 10 mms. in distal position and with the space between the premolars to be less than 6 mms. At ages 9 and 10 years, the cases seemed to be equally divided, as to those in which the second premolars had shifted more than 10 mms. and those which showed less shifting and space between the premolars. At ages 11 and 12 years, the majority of cases showed considerably less than 10 mms. distal shifting of second premolars or 6 mms. space between the premolars themselves. At ages 13 and 14 years all cases showed less than 10 mms. distal shifting of the second premolars and less than 6 mms. space between the premolars. Thus, in spite of the fact that the permanent first molar space was closed in all cases, the respective amount of shifting of the adjacent teeth responsible for the space closure in the mandible varied with the age of the patient at the time the permanent first molar was extracted. It would seem that if the first molar is extracted before the premolars erupt the molar space will close by extreme distal shifting of the premolars but only moderate mesial shifting of the second molar. If the manidbular first molar is extracted after the premolars erupt, the space is closed more by the mesial shifting of the second molars than by distal shifting of the premolars.

The space between the mandibular premolars may be due to the eruption of the second premolars in distal position as well as to the faster rate of shifting of the mandibular second premolar in comparison to the first premolar.

SUMMARY

When the space left by the extraction of maxillary first molar teeth was entirely eliminated, there was no relationship between the amount of distal shifting of the premolar teeth and the age of the child when the first molar extraction occurred. There was no increase in space shown between the first and second premolars in the maxilla.

In the mandible, the amount of distal shifting of the second premolar teeth and the amount of space between the premolars shows a direct correlation to the age of the child when the first molar was extracted, in spite of the fact that the space left by the extraction of the first molars was closed in all cases. The younger the child the greater the amount of space found between the first and second premolar teeth.